1980

The analysis of a glucose oxidase preparation for lactonase and catalase activity

Paul C. Myer
Lehigh University

CERTIFICATE OF APPROVAL

This research report is accepted and approved in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering.

10 May 1980
(date)

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ACKNOWLEDGEMENT

The author wishes to express his appreciation to Dr. Marvin Charles and Dr. Lazlo Nyiri for the support and guidance they gave during the course of this project. The author would also like to thank the National Aeronautics and Space Administration for providing the funds necessary to complete this project.

Paul Myer
May 2, 1980

DEDICATION

To my parents and family.

The Analysis of a Glucose Oxidase Preparation For Lactonase And Catalase Activity

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ABSTRACT

A glucose oxidase preparation was analyzed for lactonase and catalase activity and, in so doing, the enzymatic rate constants for these enzymes were determined. The rate constants were used in material balance equations to solve for reaction conditions of the glucose oxidase-catalyzed oxidation of glucose. The solution of the equations showed the effect the enzymes had on various substituent concentrations as well as on the pH and oxygen concentration of this system. From this data it was determined how effectively pH and oxygen concentration could be measured using dissolved oxygen and pH probes. The results show that a glucose oxidase preparation from A. niger was not suitable for a system wherein pH data was used to monitor the reaction kinetics.

CHAPTER ONE

INTRODUCTION

The glucose oxidase-catalyzed oxidation of glucose has been proposed as a reliable means for determining oxygen transfer rates in bioreactors. However, to obtain correct results, it is necessary to determine the activities of lactonase and catalase generally present in commercial preparations of glucose oxidase. The reason for this becomes apparent when one considers the details of the overall reaction (illustrated in Figure 1, below) and how the reaction rate measurements, which will be used to determine the oxygen transfer rate and coefficient, are affected by them.

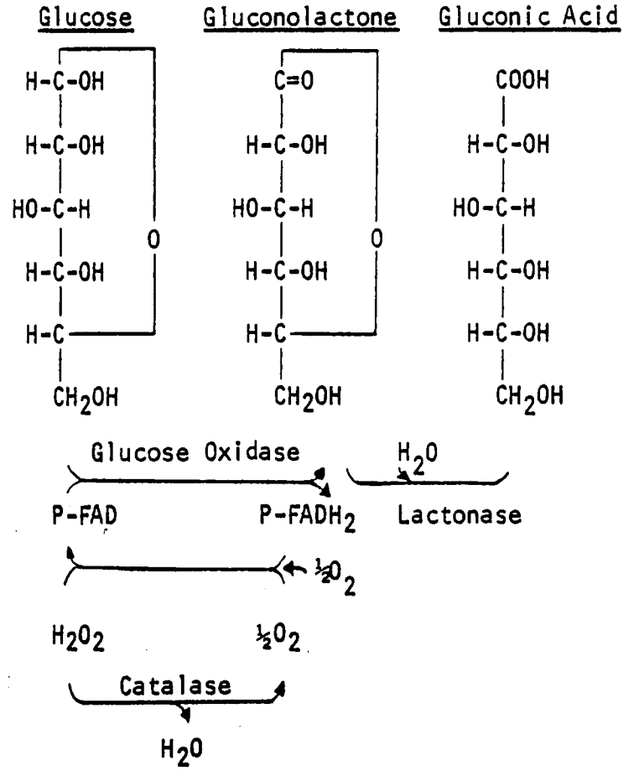

FIGURE 1. The Glucose Oxidase-Catalyzed Oxidation of Glucose

The reaction rate may be determined by any of three methods. It can be determined by measuring the transient concentrations of glucose or dissolved oxygen, or by measuring base addition to a pH-stat. However, it is clear from Figure 1 that to obtain accurate rate data, the following conditions must be satisfied. First, the glucose analysis must not be affected by the various substituents present in the reaction mixture. Second, to obtain reliable pH-stat data, it is essential that the lactone be instantaneously hydrolyzed to gluconic acid, a condition satisfied when sufficient lactonase is present in the system. Third, to develop an accurate expression for the oxygen consumption, the catalase activity, which affects the stoichiometry of the reaction, must be known accurately.

A commercial preparation of glucose oxidase, supplied by Sigma Chemical Company was assayed for lactonase and catalase activity. The preparation contained insufficient lactonase activity to insure the instantaneous hydrolysis of lactone. The catalase activity was found to be 3.7 units/ml of enzyme preparation.

The details of procedure and data analysis are presented herein.

CHAPTER TWO

THEORY AND BACKGROUND

2.1 Properties of The Enzymes

2.1.1 Glucose Oxidase*

Glucose oxidase (E.C. 1.1.3.4) catalyzes the following reaction:

$$\text{glucose} + H_2O + O_2 \longrightarrow H_2O_2 + \text{glucono-}\delta\text{-lactone}$$

Glucose oxidase is obtained from a variety of sources such as Aspergillus niger and Penicillium notatum. Glucose oxidase from A. niger is a dimer of molecular weight 186,000 which contains two FAD molecules per dimer.[1] It is stable as a solid for approximately two years at 0°C; solutions of 0.1-0.2% enzyme are stable for a week at 5°C. The enzyme becomes unstable at temperatures over 40°C.[2]

Glucose oxidases, in general, exhibit activity over a wide pH range with an optimum activity occurring at pH 5.6. Keilin and Hartree[2] have prepared an activity-pH curve as well as a temperature-activity profile for a glucose oxidase from P. notatum.

Glucose oxidase can be inhibited by glucose and hydrogen peroxide. For substrate inhibition, it was found that "at high oxygen concentrations the inhibition by glucose was not evident, but it became readily apparent at oxygen concentrations below 2×10^{-5} M (2% saturated oxygen)."[3]

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*The properties described above are for Glucose Oxidase from Aspergillus niger unless specified otherwise.

Studies [4] on hydrogen peroxide inhibition (at pH = 5.8, 25°C) indicate a 10% loss of activity when glucose oxidase is incubated for twenty minutes in a 0.2 M H_2O_2 solution. A further loss of activity (80%) occurred when glucose oxidase was incubated in 0.2 M H_2O_2 and 0.208 M glucose. Maximum loss of activity (90%) occurred when the enzyme was exposed to 0.2M H_2O_2, 0.208 M glucose and anaerobic conditions. Kleppe[4] concludes that "the inactivation is much more rapid when the flavin groups on the enzyme exist in the reduced state than when they are in the fully oxidized state."

In addition to the types of inhibition and inactivation mentioned above, there are a variety of substances whose presence results in a loss of enzyme activity. Halides have been identified as simple competitive inhibitors. The enzyme is also inhibited by Ag^{+}, Hg^{++}, and Cu^{++}.[5] Other types of inhibitors are given in the literature.[1,5]

2.1.2 Catalase*

Catalase (E.C. 1.11.1.6) catalyzes the following reaction:

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

Catalase (beef liver) has a molecular weight of approximately 250,000.[5] "All preparations are stable for six to twelve months at 5°C."[5]

Chance[6] found that the activity of catalase (horse blood) ". . . is constant in the region pH 4 to pH 8.5." Other authors[7,8]

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*The above information refers to catalases obtained from a variety of sources. The catalase source is listed in the parentheses.

have noted the complete loss of activity below pH 3 (for bovine liver catalase) and above pH 10 (for porcine blood catalase).

Catalase can lose its activity due to substrate inhibition. Bonnichsen, et. al.[9] indicates that dilute solutions of catalase (horse blood) will experience loss of activity due to hydrogen peroxide and that the effect is more pronounced at higher temperatures. However, in more concentrated catalase solutions, there is no inhibition for substrate concentrations up to 1 M.

The presence of various ions will also lead to a decrease in catalase activity. Cyanide has been identified[10] as an inhibitor as has copper (II) ions, ascorbic acid,[11] and acetate ions.[9] The literature[5] cites various other factors leading to the inactivation of catalase.

2.1.3 Gluconolactonase

Lactonase has been identified as the enzyme which catalyzes the hydrolysis of glucono-σ-lactone to gluconic acid. It is present in a number of microorganisms. Hsieh, et. al.[12] found lactonase in a crude preparation of glucose oxidase from A. niger. Lactonase has been found in extracts of Pseudomonas fluorescens[13] and in baker's yeast.[14]

According to Jermyn,[13] lactonase (from P. fluorescens) has an optimum activity at approximately pH 5.5 in a 0.5 M sodium acetate buffer. The activity is reduced (40%) at higher pH's when a collidine-HCl buffer is used. Total inhibition occurs in the presence of a phosphate buffer (pH = 5.5).

In addition to phosphate, various other materials act to inhibit lactonase. Jermyn[13] cited gluconate, xylonate, galactonate and arsenate as lactonase inhibitors. Lipmann and Brodie[14] found that their lactonase (extracted from baker's yeast) was inhibited by sodium fluoride, sodium benzoate, and hexylresorcinol at concentrations of 4×10^{-3} M.

2.2 Determining The Effect of Catalase and Lactonase on The Oxygen and Gluconic Acid Concentrations

The reactions listed below are those for a glucose solution exposed to a glucose oxidase preparation which contains lactonase and catalase.

I) glucose + O_2 + H_2O $\xrightarrow[\text{Oxidase}]{\text{Glucose}}$ H_2O_2 + glucono-δ-lactone

II) H_2O_2 $\xrightarrow{\text{Catalase}}$ $H_2O + \frac{1}{2}O_2$

III) glucono-δ-lactone + H_2O $\longrightarrow$ gluconic acid*

For reaction I a rate expression for the consumption of glucose has been derived:[15]

$$-r_{glucose} = \frac{A}{1 + \frac{\alpha}{[O_2]} + \frac{\beta}{[S]}} \qquad (1)$$

where $\alpha = 0.5 \times 10^{-3}$ M
$\beta = 0.07$ M
A = activity of the glucose oxidase**

The decomposition of H_2O_2 via catalase (reaction II) follows first order kinetics[16] (for H_2O_2 concentrations of 0.1 to 0.5 M):

---

*Reaction III occurs spontaneously or, in the presence of lactonase, both spontaneously and enzymatically.

**See Appendix E, p. 138 for information concerning the glucose oxidase activity.

$$-r_{H_2O_2} = k_c [H_2O_2] \quad (2)$$

The spontaneous and the enzymatic hydrolyses of glucono-σ-lactone are known to be first order reactions:[12,13]

$$-r_{lactone} = k_s [\text{Gluconolactone}] \quad (3)$$

$$-r_{lactone} = k_o [\text{Gluconolactone}] \quad (4)$$

where $k_o = k_e + k_s$[12]

k_o = "overall" lactone hydrolysis rate constant

k_e = rate constant for enzymatic hydrolysis

k_s = rate constant for spontaneous hydrolysis

The reaction rate constants for reactions II and III must be determined experimentally. Details of the determination are given in the experimental section on pages 18-25.

A material balance on the closed reaction system yields the following differential equations:

$$\frac{d[S]}{dt} = \frac{-A}{1 + \frac{\alpha}{[OX]} + \frac{\beta}{[S]}} \quad (5)$$

$$\frac{d[OX]}{dt} = \frac{-A}{1 + \frac{\alpha}{[OX]} + \frac{\beta}{[S]}} + \frac{k_c[HP]}{2} \quad (6)$$

$$\frac{d[HP]}{dt} = \frac{A}{1 + \frac{\alpha}{[OX]} + \frac{\beta}{[S]}} - k_c[HP] \quad (7)$$

$$\frac{d[GL]}{dt} = \frac{A}{1 + \frac{\alpha}{[OX]} + \frac{\beta}{[S]}} - k_o[GL] \quad (8)$$

$$\frac{d[GA]}{dt} = k_o[GL] \quad (9)$$

where [S] = glucose concentration, moles/liter
[OX] = oxygen concentration, moles/liter
[GL] = gluconolactone concentration, moles/liter
[GA] = gluconic acid concentration, moles/liter
k_c = rate constant for decomposition of H_2O_2 via catalase

The above equations are solved, using a CDC 6400 computer*, for different initial concentrations of glucose, oxygen, and glucose oxidase preparation.

The solution of the differential equations gives concentration history data for the reaction components at various times. The data will allow comparison of an oxygen concentration curve for a system containing catalase with one that is produced by a catalase-free system. From this observation, it can be determined to what extent catalase (in the amount found in the glucose oxidase preparation) affects the oxygen concentration, and whether a dissolved oxygen probe can be used effectively to monitor reaction rates for this particular system. Also, the data will permit a comparison between the rates of lactone hydrolysis and glucose oxidation. If sufficient lactonase is present in the glucose oxidase preparation, the rate of lactone hydrolysis will predominate allowing the reaction rate of this system to be monitored via a pH probe.

2.3 Methods For Determining Catalase Activity

Catalase activity (for solutions containing catalase) is expressed in terms of units per milliliter. According to Sigma,

*Lehigh University Computing Center.

a unit is defined as the following:

> One unit will decompose 1.0 μmole of H_2O_2 per minute at pH 7.0 at 25°C while the H_2O_2 concentration falls from 10.3 to 9.2 μmoles per ml of reaction mix.

Several procedures, based on measuring the rate of decomposition of hydrogen peroxide, have been proposed for determining catalase activity. One method calls for measuring hydrogen peroxide concentrations titrimetrically, using either standardized potassium permanganate[17] or sodium thiosulfate[18] solutions. A somewhat simpler technique monitors the change in hydrogen peroxide concentration spectrophotometrically.[19] All of the methods measure the rate of the enzyme-catalyzed decomposition of hydrogen peroxide at pH 7.0 and 25°C. These methods are examined in the experimental section of this report.

2.4 Determining The Reaction Rate Constants For

i) H_2O_2 Decomposition Via Catalase

ii) Lactone Hydrolysis: Spontaneous and Enzymatic

2.4.1 Determining The Rate Constant For The Decomposition of Hydrogen Peroxide

According to Aebi,[16] "the decomposition of H_2O_2 initially (ca. 0-30 seconds) follows that of a first order reaction with H_2O_2 concentrations between 0.01 and 0.05 M. The rate constant (k) for the overall reaction is given by:

$$k = \frac{1}{\Delta t} \ln \frac{S_1}{S_2} \qquad (10)$$

where $\Delta t = t_2 - t_1$ = measured time interval; S_1 and S_2 = H_2O_2 concentrations at times t_1 and t_2."

Beers and Sizer[19] measured the rate constants* for solutions containing different concentrations of catalase and found that the decomposition of H_2O_2 followed first order kinetics for periods longer than ninety seconds.

In the present study, the reaction rate constant is determined using equation (10), but for a reaction time of ninety seconds. The procedure is listed on page 18.

2.4.2 Determining The Rate Constants For Lactone Hydrolysis

Gluconolactone is hydrolyzed spontaneously or, in the presence of lactonase, both spontaneously and enzymatically.

The rate constants for these reactions have been determined using a number of techniques. Hsieh, et. al.[12] used a pH-stat to determine the rates of hydrolysis by measuring the amount of sodium hydroxide needed to maintain a constant pH. Lien[20] measured the rate of hydrolysis by monitoring, spectrophotometrically, changes in lactone concentration. In both cases, the rate constants were determined at pH 5.5 and 30°C.

A modified version of Lien's method is developed in the experimental section.

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*The rate constants were determined for pH 7.0 (.01 M phosphate buffer), 25.5°C, and over an H_2O_2 concentration range of .015 - .005 M.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Developing an Assay For Determining Catalase Activity

Catalase activity in a commercial preparation of glucose oxidase was measured by several methods, each based on monitoring the rate of decomposition of hydrogen peroxide. The rate of hydrogen peroxide decomposition was measured either by 1) titration, using a potassium permanganate or iodometric titration, or 2) spectrophotometric determination of hydrogen peroxide at 240 nm.

The spectrophotometric method provided the simplest and most accurate means of determining catalase activity. The titrimetric methods were unsuitable because of the occurrence of side reactions. Details of both procedures are given below.

3.1.1 Determining Catalase Activity: Measuring Changes in Hydrogen Peroxide Concentration Using a Potassium Permanganate Titration

Catalase activity was measured by using a potassium permanganate titration to monitor changes in hydrogen peroxide concentration. The method, first described by Waksman[17], is simple: A glucose oxidase preparation (or any substance suspected of containing catalase) is added to a hydrogen peroxide solution (approximately 0.1 M). The solution is mixed thoroughly and maintained at 25°C and pH 7.0 (using a .05 M phosphate buffer). At various times samples are withdrawn, heated to boiling to deactivate the enzyme, and titrated with a standardized potassium permanganate solution. The resulting hydrogen peroxide data is

used to determine the catalase activity* by measuring the number of micromoles of hydrogen peroxide decomposed per minute per ml of enzyme preparation.

The above method was found to be unsuitable for determining hydrogen peroxide concentrations in the presence of the glucose oxidase preparation. Specifically, the following difficulties were encountered:

1) The titration endpoint was unstable because the potassium permanganate oxidized enzymes in the glucose oxidase preparation.
2) The glucose oxidase preparation caused foaming, thereby making it very difficult to obtain accurate titration data.
3) The enzyme preparation imparted a yellow color to the titration solution which further complicated the task of finding the endpoint.

The measurement of hydrogen peroxide concentrations via iodometric titration[18] might not have been plagued with so many problems because it contained no oxidants stronger than hydrogen peroxide. However, it was not evaluated because foaming and endpoint-color interference would most likely have occurred.

3.1.2 Spectrophotometric Determination of Catalase Activity

3.1.2.1 Introduction

Very low concentrations of hydrogen peroxide (below inhibitory level) can be measured spectrophotometrically at 240 nm.

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*See p. 10 for the definition of catalase activity.

A spectrophotometric assay can be used to determine the catalase activity as follows: A catalase-containing material is dissolved in a solution of hydrogen peroxide and the time required for the hydrogen peroxide concentration to go from 10.3 μmoles/ml to 9.2 μmoles/ml is measured. The assay is performed at 25°C and pH 7.0* (refer to catalase activity definition, p. 10).

To facilitate the use of the spectrophotometric assay, the H_2O_2 concentrations are converted to absorbances by using the molar extinction coefficient of H_2O_2. A procedure used to determine the molar extinction coefficient of H_2O_2 (at 240 nm) is listed on page 15.

3.1.2.2 Materials

Sigma's glucose oxidase preparation (from Aspergillus niger) was assayed for catalase activity and, also, used for the rate constant determination (see Section 3.2, p. 18).

The spectrophotometer used was a Beckman Model 25** equipped with jacketed cuvettes. The cuvettes, as well as several enzyme solutions, were maintained at a constant temperature by a Masterline Model 2800 water bath (Forma Scientific).

For the catalase rate constant determination (section 3.2), a push-button syringe (Hamilton, Model CR 700-200) was used for rapid injection of H_2O_2 solutions into the enzyme solution.

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*The catalase activity ". . . is constant in the region pH 4 to pH 8.5." See reference 6.

**The Beckman Model 25 spectrophotometer was borrowed from the Department of Chemistry, Lehigh University.

3.1.2.3 Determining The Molar Extinction Coefficient of H_2O_2 at pH = 7.0, 25°C and 240 nm

A 1/100th dilution of a 30% H_2O_2 solution was prepared in a .05 M phosphate buffer. The exact H_2O_2 concentration of this solution was determined titrimetrically using a standardized 0.1 N $KMnO_4$ solution (see pp. 71-72 of Appendix A for procedures relating to the preparation and standardization of both H_2O_2 and $KMnO_4$ solutions). Dilutions (1/4, 1/5, 1/8, 1/10) of the standardized solution were prepared in .05 M phosphate buffer (pH = 7.0) and placed in the constant temperature bath (25°C).

After one hour in the bath, each solution was transferred to a jacketed cuvette and its absorbance determined at 240 nm. The resultant absorbance data is listed in Table 2, page 77.

A graph of the absorbance versus concentration data is shown on page 78; the slope of the straight line on this graph is the value for the molar extinction coefficient.

3.1.2.4 Developing An Assay For Determining Catalase Activity

Sigma's suggested assay (see page 140 of Appendix E) for relatively pure catalase preparations proved to be inadequate for measuring catalase activity in glucose oxidase preparations, and a slightly modified procedure was developed. In the course of developing this procedure, several things became apparent:

1) The glucose oxidase preparation was too dark in color to assay with only one cuvette (as suggested by the Sigma procedure). The effect of the dark color had

to be "blanked out" by using both reference and sample cuvettes.

2) Because of the color of the enzyme preparation, it was essential that the concentration of enzyme in both cuvettes was exactly the same.

3) Since Mohr pipettes were used to transfer the various materials into the cuvette, it was important that, for each separate addition, as large a sample as possible was transferred so as to reduce the relative pipetting error.

Taking into account all of these considerations, the following assay was devised:

Catalase Activity Determination

1) Prepare a 1/500th dilution (approximately) of the stock H_2O_2 (30%) solution by pipetting 0.2 ml stock H_2O_2 into a 100 ml volumetric flask and adding approximately 90 ml of .05 M phosphate buffer (pH = 7.0).

2) Prepare a buffered enzyme solution by pipetting 1.0 ml of Sigma glucose oxidase preparation into a 10 ml volumetric flask and diluting to the mark with .05 M phosphate buffer.

3) Place the solutions from steps 1 and 2 into a constant temperature bath maintained at 25°C. (A third flask containing .05 M phosphate buffer is also placed in the bath). The flasks are kept in

the bath for one hour. When the solutions have reached a stable temperature of 25°C, proceed with the following steps.

4) In a clean, dry "reference" cuvette (jacketed--1 ml total volume), mix 0.5 ml of buffered enzyme solution with 0.5 ml .05 M phosphate buffer. The solutions are transferred from the flasks (within the constant temperature bath) to the cuvette using Mohr pipettes.

5) To a clean, dry "sample" cuvette (jacketed--1 ml total volume), mix 0.5 ml buffered enzyme solution with 0.5 ml of the H_2O_2/phosphate buffer solution (from step 1).

6) Note the time it takes for the absorbance to go from a value of A_{240} = .413 to A_{240} = .368.* These absorbances correspond to H_2O_2 concentrations of 10.3 μmoles/ml and 9.2 μmoles/ml, respectively.

A discussion of the results and interpretation of this assay is given in "Results and Discussion" starting on page 29.

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*The timer is started as soon as a value of A_{240} = .413 appears on the spectrophotometer's display. The timer is stopped as soon as A_{240} = .368 appears on the spectrophotometer's display.

3.2 Determining The Rate Constant For Decomposition of H_2O_2 Via Catalase

A procedure similar to the catalase activity assay is used to determine the first order rate constant. The procedure follows:

1) Prepare a 20% dilution of the Sigma glucose oxidase preparation in a sodium acetate buffer (pH = 5.5). Place the solution in the constant temperature bath ($30^{o}C$).
2) After one hour in the bath, transfer one ml of the solution to a "reference" cuvette and another one ml to a "sample" cuvette. Both cuvettes are jacketed and each has a capacity of about 1 ml.
3) Using the push-button syringe, inject roughly 2 µl of 30% H_2O_2 solution into the sample cuvette.
4) Mix the contents of the sample cuvette rapidly and thoroughly. Place both cuvettes in the spectrophotometer, start the timer, and take an initial absorbance reading.
5) Let the reaction proceed for 90 seconds. At t = 90 seconds, take a second absorbance reading. Using the absorbance-time data, calculate k where

$$k = \frac{1}{\Delta t} \ln \frac{A_1}{A_2} \qquad \Delta t = t_2 - t_1$$

The data and the calculated rate constant are listed on pages 81 and 82 (Appendix B).

3.3 Determining The Rate Constants For The Spontaneous And Enzymatic Hydrolysis of Glucono-δ-Lactone

3.3.0.1 Introduction

Both the enzymatic and the spontaneous hydrolyses of glucono-δ-lactone are first order reactions. The rate constants for these reactions were determined by measuring the change in lactone concentration with time for lactone solutions containing different amounts of lactonase. The reaction rate constants were determined at pH = 5.5 and 30°C.

Lien's method[20] was used to determine the lactone concentration history. This method involves reacting the lactone with hydroxylamine to form hydroxamic acid. Subsequent treatment of the hydroxamic acid with ferric chloride results in an iron (III)/hydroxamic acid complex which absorbs radiation at 540 nm. Measuring the absorbance of this complex will indicate the lactone concentration.

Before the rate constants for lactone hydrolysis could be determined, it was necessary first to develop a lactone calibration curve and, second, to find a buffer system (pH = 5.5) which neither inhibits lactonase nor interferes with Lien's method of gluconolactone analysis. Methods for performing all of these tasks are described in the following pages.

3.3.0.2 Materials

A glucose oxidase enzyme preparation (Sigma Chemical Company) obtained from Aspergillus niger was used in the studies of enzymatic hydrolysis of glucono-δ-lactone. Information

concerning this preparation is given in the Appendix, pages 138-139.

The gluconolactone solutions were prepared from glucono-σ-lactone powder (obtained from Sigma Chemical Company). Because of its high purity (99.7%), this reagent was used to prepare the gluconolactone calibration curve.

Other reagents used for the assay include:

Hydroxylamine reagent--Hydroxylamine reagent is prepared by mixing equal volumes of 4 M NaOH and 4 M hydroxylamine hydrochloride. The pH of the mixture is adjusted to pH = 8.0 using the appropriate component. This solution should be prepared immediately before using.

Ferric chloride reagent--This reagent is prepared by dissolving 20 grams $FeCl_3 \cdot 6H_2O$ in 0.1 N HCl and diluting to 100 ml total volume.

4 M HCl--33 ml concentrated HCl is dissolved in water and diluted to 100 ml total volume.

The buffers considered for use in the lactone hydrolysis assay are the following: sodium acetate-acetic acid, potassium hydrogen phthalate-sodium hydroxide, and citric acid-sodium phosphate dibasic. The preparation of these buffers for pH = 5.5 is described in the Appendix, pages 72-73.

A Bausch and Lomb Spectronic 700 spectrophotometer, equipped with silica cuvettes, is used to measure the absorbances of the iron (III)/hydroxamate solutions at 540 nm.

During the assay, transfer of solutions is accomplished using a 1.0 ml adjustable syringe (Clay Adams).

3.3.1 Preparation of a Glucono-σ-Lactone Calibration Curve

A 4.36×10^{-2} M hydroxamate solution was prepared by dissolving 7.7652 grams of glucono-σ-lactone* in the hydroxylamine reagent** and diluting to one liter. From this "stock" solution, dilutions (1/100, 2/100, 3/100, 4/100, 5/100) in hydroxylamine reagent were prepared and subjected to the following spectrophotometric assay:

For the sample: Mix together in a test tube 5.5 ml of hydroxamate solution and 2.0 ml 4 M HCl. Place tube and contents in a water bath (ambient temperature) for ten minutes. After ten minutes, add 4.5 ml ferric chloride reagent.

For the blank: Mix together in a test tube 5.5 ml of hydroxylamine reagent and 2.0 ml 4 M HCl. Place tube and contents in a water bath (ambient temperature) for ten minutes. Afterwards, add 4.5 ml ferric chloride reagent.

Note: The pH of the final mixture (after adding the ferric chloride reagent) should be within pH = 1.2 ± 0.2 (according to Lien[20]). However, the pH did not have to be restricted to this range since later experiments revealed no adverse effects when the solutions had a pH of 0.5-1.2. Problems (precipitate formation) were encountered when the pH of the assay mixture was pH $\geq$ 1.4.

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*No preliminary drying of the glucono-σ-lactone was necessary since its moisture content was 0.048%.

**Cori and Lipmann[21] note that "the alkaline hydroxylamine reagent . . . gave a practically instantaneous complete conversion of gluconolactone into hydroxamic acid."

The absorbances of the hydroxamate solutions were determined at 540 nm. Table 6, page 83, lists the absorbance concentration data for each of the hydroxamate solutions.

A gluconolactone calibration curve* was prepared from the data of Table 6 and is shown on page 84. From this curve, the molar extinction coefficient for the iron (III)/hydroxamic acid complex was found to be $\epsilon_{540} = 935.0\ l cm^{-1} mole^{-1}$.

3.3.2 Evaluation of Various Buffers For The Lactone Hydrolysis Assay

A variety of buffer systems can be used to maintain solutions at pH = 5.5. However, to be used for the lactone hydrolysis assay (Lien's[20] method), the particular buffer system must satisfy the following criteria:

1) The buffer must not inhibit lactonase.

2) The buffer components must not react with either the hydroxylamine reagent or with glucono-δ-lactone.

3) The buffer components must not precipitate when the pH of the solution is lowered to approximately pH = 1.0 (as called for in Lien's method; see note on page 21).

4) The ionic species present in the buffer system must not interfere with the iron (III)/hydroxamate complex.

The following buffer systems were evaluated: citric acid-sodium phosphate dibasic, and potassium hydrogen phthalate-sodium hydroxide.

---

*Since there was a "complete conversion of gluconolactone into hydroxamic acid"[21] the lactone concentration is directly related to the hydroxamic acid concentration on a one-to-one basis.

Preliminary tests proved the potassium hydrogen phthalate-NaOH buffer was unsuitable because of precipitate formation at low pH (pH~1.0); the citric acid-phosphate buffer showed no signs of precipitate formation.

Further tests* indicated that the citric acid-phosphate buffer could be used for Lien's[20] method of determining lactone concentrations; that is, the citric acid-phosphate buffer satisfied conditions two and four. However, Jermyn[13] points out that a lactonase (obtained from Pseudomonas fluorescens) ". . . was strongly inhibited by phosphate ions . . ." and for this reason, the citric acid-phosphate buffer was no longer considered for the lactone hydrolysis assay. Instead, a 0.5 M sodium acetate buffer was used, primarily because it had been used with success by Jermyn[13].

3.3.3 Developing an Assay Procedure For Measuring Changes in Glucono-σ-Lactone Concentration

A procedure has been developed for measuring rates of hydrolysis of glucono-σ-lactone. The procedure is listed below.

Using the Sigma glucose oxidase preparation, prepare 0%, 4%, and 8% enzyme solutions in 0.5 M sodium acetate buffer (pH = 5.5). Each of the enzyme solutions is analyzed as follows:

1) Prepare two of the following: Place a magnetic stirring bar into a 125 ml erlenmeyer flask. Into the flask, pipet 25.0 ml of the enzyme solution. Cover the mouth

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*See the Appendix (p.73) for the procedure used to evaluate the various buffers.

of the flask and place it in a constant temperature bath maintained at 30°C.

2) Into a 25 ml volumetric flask, add enough enzyme solution to fill the flask to the mark. To this add 0.5 ml water. Mix contents thoroughly, and place the flask in a constant temperature bath (T = 30°C). This solution is the "blank" solution.

3) Allow the solutions from steps one and two to reach 30°C. The solutions were kept in the bath for approximately 45 minutes.

4) Prepare a glucono-δ-lactone solution by dissolving 2.5-3.0 grams of glucono-δ-lactone in approximately 50 ml of water. (Use this solution soon after preparation since solutions of glucono-δ-lactone hydrolyze spontaneously.)

5) Remove the flask, containing the enzyme solution, from the constant temperature bath, and place it on a magnetic stirrer. Stir the flask's contents moderately to avoid splashing. To the enzyme solution, add 0.5 ml of the glucono-δ-lactone solution (from step 4). The lactone solution is added via a syringe. The instant the lactone solution is added, a timer is started.

6) After mixing the enzyme/lactone mixture for approximately 15 seconds, return the flask and its contents to the constant temperature bath.

7) At different times, remove 1.0 ml samples (using a 1.0 ml syringe) from the enzyme/lactone solution and place them into test tubes containing 4.0 ml hydroxylamine reagent. A separate test tube is used for each different sample. Mix the contents of each test tube thoroughly.

8) To a separate test tube labeled "blank", add 4.0 ml hydroxylamine reagent to 1.0 ml of the "blank" solution prepared in step 2. Mix contents.

9) To the various "sample" test tubes and the "blank" test tube, add 2.0 ml 4 M HCl. Mix contents of the test tubes thoroughly, and place the solutions in a water bath (ambient temperature) for about ten minutes.

10) After ten minutes, add 1.0 ml ferric chloride reagent to each of the test tubes. Mix the contents thoroughly, and analyze the various solutions spectrophotometrically at 540 nm. The absorbances of the various solutions must be determined within ten minutes from the time the ferric chloride reagent is added. The reason for this is because the color of the solution fades with time.

An analysis of the above assay is given in "Results and Discussion," pages 32-35.

3.4.0 Determining The Effect of The Glucose Oxidase Reaction Products on The YSI Glucose Analyzer

3.4.0.1 Introduction

The reaction rate of the glucose oxidase-catalyzed oxidation of glucose can be determined by periodically sampling the reaction mixture and measuring the change in glucose concentration with time.

The apparatus used for measuring glucose concentrations is the YSI* Glucose Analyzer (model 23A). Briefly, the instrument measures glucose concentration as follows: A solution containing glucose is injected into the analyzer where it comes in contact with a glucose oxidase-impregnated membrane. The immobilized glucose oxidase converts the glucose to H_2O_2 and gluconic acid via the following reaction -

$$\text{glucose} + O_2 \xrightarrow[\text{oxidase}]{\text{glucose}} \text{gluconic acid} + H_2O_2$$

The hydrogen peroxide concentration is measured within the analyzer via a platinum/silver electrode. The resulting concentration data is displayed in terms of mg/dl of glucose.

Solutions from the glucose oxidase reaction system can be analyzed for glucose concentration using the glucose analyzer. However, to obtain accurate glucose concentration data, it is important that reaction substituents such as gluconic acid, H_2O_2, and gluconolactone do not interfere with the glucose analyzer. A procedure for determining the effect that these compounds have on the glucose analyzer is given on page 27.

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*Yellow Springs Instrument Company.

3.4.0.2 Materials

The glucose analyzer (Yellow Springs Instrument) was used to measure glucose concentrations up to 200 mg/dl (.011 M). The analyzer was supplied with a 200 mg/dl glucose solution, for use in standardizing the instrument. A syringe (YSI model 2361) was used to inject samples into the analyzer.

Gluconolactone solutions of .01, 0.1, and 1.0 M were prepared from gluconolactone powder (Sigma Chemical Company). Gluconic acid solutions (10% and 25%) were prepared by appropriate dilutions of a 50% gluconic acid solution (Eastman Chemicals). Likewise, H_2O_2 solutions (.011, 5.5×10^{-3} M) were prepared from a 30% H_2O_2 solution. The exact concentrations of the H_2O_2 solutions were determined by potassium permanganate titration (see Appendix A, page 71).

3.4.0.3 Procedure

The glucose analyzer was calibrated and operated according to procedures outlined in the YSI Glucose Analyzer Instruction Manual[22].

Once the glucose analyzer was calibrated, the different solutions were injected into the analyzer. Each solution was analyzed twice; after every fourth injection, the analyzer was recalibrated.

The results of the above procedure are listed in Table 9, page 91. A discussion of how the various solutions affect the glucose analyzer is given on pages 68-69.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Spectrophotometric Measurement of The Catalase Activity and Rate Constant in The Sigma Glucose Oxidase Preparation

4.1.1 Determination of The Molar Extinction Coefficient of H_2O_2 (at pH = 7.0, 25°C)

The concentration/absorbance data for the various H_2O_2 solutions is listed in Table 2, page 77. From this data, the molar extinction coefficient was calculated to be 40.05 l/cm mole (at 240 nm).

Beers and Sizer[19] have found the molar extinction coefficient of H_2O_2 to be 39.8 l/cm mole while Sigma suggests a value of 43.6 $\frac{1}{\text{cm mole}}$.* All of the molar extinction coefficients were determined at 240 nm.

Using the molar extinction coefficient of 40.05 l/cm mole, absorbance values corresponding to H_2O_2 concentrations of 10.3 $\frac{\mu\text{moles}}{\text{ml}}$ and 9.2 $\frac{\mu\text{moles}}{\text{ml}}$ were calculated. The absorbance values were A = .413 and A = .368, respectively. It was over this H_2O_2 concentration range that the catalase activity was determined (see page 16 for the assay).

An analysis of the data used in calculating the molar extinction coefficient is given in the Appendix, page 76.

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*Refer to the Sigma catalase activity assay in Appendix E, page 140.

4.1.2 Evaluating The Catalase Activity Assay

According to the assay listed on page 16, the Sigma glucose oxidase preparation contained a catalase activity of 3.7 units/ml. Sigma assayed the same preparation and found a catalase activity of 9.4 units/ml. The difference in activities can be attributed to the different times at which this particular lot (lot number 97C-0322) was assayed; a period of fifteen months had elapsed from the time Sigma assayed it to when it was analyzed as per the analysis of page 16.* Over this time period, the enzyme probably lost some of its activity. Also, any adverse conditions experienced during shipment may have caused it to become further deactivated.

Data obtained from the catalase activity determination are listed in Table 3, page 79. The data indicates the assay can be done with relatively good precision. The maximum percent deviation from the mean is 4.4% (see Table 4, page 80).

Differences in the activity values resulted from difficulties encountered with timing the reaction. Both the starting and stopping points were somewhat arbitrary due to randomly fluctuating absorbance values. These random fluctuations, apparently caused by oxygen bubble agglomeration in the cuvette and the presence of suspended particles in the enzyme preparation, created "uncertainty

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*See page 138 for information concerning the date Sigma assayed the glucose oxidase preparation.

periods"* of approximately fifteen seconds each. To reduce the error, the overall reaction time was increased by lowering the catalase concentration in the cuvette. In addition, the problem of oxygen bubble agglomeration was alleviated somewhat by incorporating the following precautions into the assay:

1) The contents of the cuvettes were gently mixed to avoid foaming.
2) A dilute solution of enzyme preparation was analyzed. The diluted enzyme solution did not trap oxygen bubbles as readily as more concentrated solutions.
3) Both the sample and reference cuvettes were cleaned with a chromic acid solution immediately before doing the assay.

The problem of suspended particles could (possibly) be eliminated by centrifugation or more elaborate methods (protein precipitation). However, these methods were considered inappropriate for an assay intended to be as simple as possible and, therefore, were not investigated.

An analysis of the catalase activity assay and how its precision is affected by the use of Mohr pipettes is shown on pages 95-96.

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*The "uncertainty period" was regarded as the time interval during which the "starting" absorbance value (A = .413), (or final absorbance A = .368), constantly reappeared on the spectrophotometer's display after the timer was started (or stopped).

4.1.3 Evaluation of The Assay For Determining The Rate Constant of The Decomposition of H_2O_2 Via Catalase

The results from the catalase rate constant determination (page 18) are shown in Table 5, page 81. Six absorbance values (three initial and three final) were used for obtaining average initial and final absorbances which, in turn, were used to calculate the rate constant. This method of averaging the absorbance values was used so as to overcome the problem of randomly fluctuating absorbance values.*

The rate constant for the decomposition of H_2O_2 via catalase was found to be $k = 0.399\ min^{-1}$ at pH = 5.5 and 30°C. For the purpose of comparison, a rate constant was calculated using a formula given by Aebi[16]. The "calculated" rate constant was found to be $k = .755\ min^{-1}$. (See Appendix E, pages 142-143.)

The precision obtained by this assay was good; the maximum percent deviation from the mean was 4.5%. As in the catalase activity assay, oxygen bubble agglomeration and suspended particulate matter affected the precision. Ways of eliminating these problems were discussed previously (see page 30).

Pipetting errors did not have a significant effect on the assay precision: enzyme solutions in the reference and sample cuvettes were identical having come from the same stock solution; the

*Random fluctuations in the absorbance values, apparently caused by O_2 bubble agglomeration, made it difficult to determine the initial and final absorbance values. Rather than guessing which absorbance value was the correct initial (final) value, a mean absorbance value was obtained by averaging the first three absorbance values which appeared after the timer was started (stopped). The three initial (final) absorbance values were taken in less than two seconds.

addition of 2 µl of H_2O_2 to the sample cuvette changed its enzyme concentration by 0.2%--a negligible amount; changes in H_2O_2 concentration were observed rather than absolute H_2O_2 concentrations.

4.2 The Spontaneous and Enzymatic Hydrolysis of Gluconolactone

4.2.1 Determination of the Rate Constants

The rate constant for the spontaneous hydrolysis of gluconolactone, as determined by using the assay of page 23, was found to be .0178 min^{-1}. Jermyn[13], using a similar method, found the rate constant to be .0181 min^{-1}. Both determinations were made for a solution consisting of gluconolactone (initial concentration $\sim 9.0 \times 10^{-5}$ M) in a 0.5 M sodium acetate buffer (pH = 5.5) at a temperature of 30°C. The relative error of the experimental value from Jermyn's value is -1.66%.

The rate constants for the enzymatic hydrolysis of gluconolactone via lactonase were determined for a 4% (4 ml glucose oxidase preparation diluted to 100 ml with 0.5 M sodium acetate buffer, pH = 5.5) and an 8% enzyme solution. The results of the tests are listed in Table 7, page 85. From this data the rate constants for the various enzyme solutions were calculated (see Table 8, page 90).

The data from Table 8 was used to calculate the enzymatic rate constant for the undiluted glucose oxidase preparation. The enzymatic rate constant was found to be $k_e = 1.158\ min^{-1}$. Combining the enzymatic rate constant (k_e) with the spontaneous rate constant

(k_s) yields the overall rate constant (k_o) where:

$$k_o = k_s + Dk_e = .0178\ min^{-1} + 1.158(D)\ min^{-1}$$

(D) is a dilution factor.

The precision obtained using the method of page 23 was good despite the amount of pipetting done during the assay. For the rate constant determinations involving the 0%, 4%, and 8% enzyme solutions, the deviations from the mean were 1.1%, 4.6%, and 1.3%, respectively. An analysis shown on page 96 indicates that as much as 1.48% deviation from the mean can result when Mohr pipettes are used for this assay. Despite this potential for error, Mohr pipettes were used because they were more convenient than volumetric pipettes. Also, better precision could be obtained when the pipettes were used with reasonable care.*

4.2.2 Comparing The Lactonase Content of Different Glucose Oxidase Preparations

The rate constant/concentration data of Table 8, page 90, was put into a least squares fit program and from it the slope $\left(\frac{dk}{dc}\right)$ of the resultant line was obtained.. For the Sigma glucose oxidase preparation, the slope of the straight line was $\frac{dk}{dc} = 4.2 \times 10^{-7}\ min^{-1} \Big/ \left(\frac{\text{units g. oxidase}}{1}\right)$. Hsieh, et al.[12], using a glucose oxidase preparation obtained from Nutritional Biochemicals (NB) found the lactonase content to be such that $\frac{dk}{dc} = 1.22 \times 10^{-3}\ min^{-1} \Big/ \left(\frac{\text{units g. oxidase}}{1}\right)$.

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*Table 10, page 93, shows the precision and accuracy of a Mohr pipette when used to deliver 1.0 ml volumes.

Hsieh, et al.[12]; reported that the NB preparation was suitable for a system whereby the kinetics could be monitored via pH and dissolved oxygen probes; ". . . the enzyme preparation has been shown . . . to contain sufficient lactonase so there is negligible accumulation of D-glucono-δ-lactone and the rate of acid production is quantitatively related to the rate of oxygen uptake." In contrast, the Sigma preparation appears to be so deficient in lactonase that pH data cannot be used to supplement the dissolved oxygen kinetic data obtained from this system. Results discussed later in the text (see pages 53-55) verify this conclusion.

4.2.3 Inhibition of Lactonase by Phosphate Ions

Using the data of Table 7, plots of the log of the lactone concentration versus time were made for the various enzyme solutions. The data points fall along a straight line, indicating first order reaction kinetics. See Figures 28-30 (pages 87-89) for the graphs.

Figures 31 through 33 on pages 145-147 show similar plots for the gluconolactone hydrolysis occurring in a citric acid-phosphate buffer, at pH = 5.5 and 30°C.*

As indicated by the graphs, there are marked differences in the rates of hydrolysis for the enzyme solutions prepared in the different buffers. The enzymatic rate constants for the 4% and 8% enzyme solutions, prepared in 0.5 M sodium acetate buffer, were .0478 min^{-1} and .0941 min^{-1}, respectively. In contrast, the enzymatic rate constants for the 4% and 8% enzyme solutions

*The experimental data is listed in Table 49, page 144.

prepared in the citric acid/phosphate buffer were .0055 min^{-1} and .013 min^{-1}, respectively. The enzymatic rate constants are approximately eight times greater in the sodium acetate buffer than in the citric acid/phosphate buffer. These data support the observation by Jermyn[13] that the enzymatic hydrolysis of gluconolactone ". . . was strongly inhibited by phosphate ions . . . ."

4.3 The Concentration History of Oxygen, Gluconolactone, and Gluconic Acid in The Glucose Oxidase Reaction System

4.3.1 Solving The Material Balance Equations For The Closed Glucose Oxidase Reaction System

The equations describing the material balance for a 300 ml closed glucose oxidase reaction system are:

$$\frac{d[S]}{dt} = \left[\frac{-A}{1 + \frac{\alpha}{[O_2]} + \frac{\beta}{[S]}}\right]\frac{X}{300} \tag{11}$$

$$\frac{d[O_2]}{dt} = \left[\frac{-A}{1 + \frac{\alpha}{[O_2]} + \frac{\beta}{[S]}} + \frac{k_c[H_2O_2]}{2}\right]\frac{X}{300} \tag{12}$$

$$\frac{d[H_2O_2]}{dt} = \left[\frac{A}{1 + \frac{\alpha}{[O_2]} + \frac{\beta}{[S]}} - k_c[H_2O_2]\right]\frac{X}{300} \tag{13}$$

$$\frac{d[GL]}{dt} = \left[\frac{A}{1 + \frac{\alpha}{[O_2]} + \frac{\beta}{[S]}}\right]\frac{X}{300} - k_o[GL] \tag{14}$$

$$\frac{d[GA]}{dt} = k_o[GL] \tag{15}$$

where $[S]^*$ = glucose concentration, M
$[GL]$ = gluconolactone concentration, M
$[GA]$ = gluconic acid concentration, M
A = activity of glucose oxidase = 2750 $\frac{\text{units}}{\text{ml}}$ = 2.75 $\frac{\text{mole}}{\text{1 min}}$
α = 0.5×10^{-3} mole/liter
β = .07 moles/liter
X = quantity (in milliliters) of glucose oxidase preparation
$k_o = k_s + k_e \frac{X}{300}$

The rate constants (see pages 31-33) are:

$k_c = .399 \text{ min}^{-1}$

$k_s = .0178 \text{ min}^{-1}$

$k_e = 1.158 \text{ min}^{-1}$

The material balance equations were solved via computer.**

However, before such a solution could be attempted, it was necessary to, first, define the initial concentrations of the various components. The initial concentrations were defined as follows: $[H_2O_2]_o = 0$ M

$[GA]_o = 0$ M

$[GL]_o = 0$ M

$[O_2]_o = 2.5 \times 10^{-4}$ M, 1.25×10^{-3} M, 2.5×10^{-3} M

$[S]_o = 1.0$ M, 0.1 M. .01 M

Three different initial concentrations were used for both glucose and oxygen. In addition, the quantity of enzyme (X) was varied as follows: X = 0.1 ml, 1.0 ml, 10.0 ml.

*Throughout the text, S will be used to denote glucose concentration (M), X will indicate quantity of enzyme preparation (milliliters), and O_2 will indicate oxygen concentration (M).

**See reference 23 for information regarding the computer solution of simultaneous differential equations.

The equations (11-15) were solved using a CDC 6400 computer and the substituent concentrations, at various times during the reaction, were obtained. Tables 13 through 39 (see Appendix D, pages 101-127) list the concentration history of the various components for the different initial conditions.

Equations 11-15 were also solved for a system which contained no catalase (i.e., $k_c = 0$).* Tables 40 through 48 list the concentration data for the various substituents in a catalase-free system.

A discussion of the various systems and how they are affected by the catalase and lactonase activities, as well as initial glucose and oxygen concentrations, is given in the following sections.

4.3.2 Oxygen Concentration History: Comparing Oxygen Concentrations For a System Containing Catalase With One That is Catalase-Free

The oxygen concentration histories for reaction systems containing different quantities of catalase and glucose oxidase are shown in Tables 13-39, pages 101-127. The oxygen concentration histories for similar systems containing no catalase are shown in Tables 40-48, pages 128-136. In all cases, the systems are closed and operating at temperatures of 30°C and pH = 5.5. The enzyme activities and initial reactant conditions are listed on each of the tables.

Comparing the computer-generated oxygen concentration data for two systems with identical initial conditions, it can be seen

*The computer solution for the catalase-free system was done for only one initial glucose concentration, $S_o = 0.1$ M.

that oxygen reaches a much lower concentration in a catalase-free system than in a catalase-containing system. For example, in a catalase-free system, with initial conditions of O_{2o} = 2.5 x 10^{-3} M, S_o = 0.1 M and X = 1.0 ml, the oxygen concentration (after three minutes) decreases to a level of 1.63 x 10^{-23} M (see Table 45). In contrast, for the same reaction time, a catalase-containing system with identical initial reactant conditions has an oxygen concentration of 9.05 x 10^{-8} M (see Table 27). The oxygen concentrations for the two systems differ (as expected since there is no generation of O_2 in the catalase-free system) by a factor of approximately 2 x 10^{-14}.

The computer data for oxygen indicates a tremendous difference between the oxygen concentrations for the two systems. Whether or not an actual reactor system could distinguish between these concentration differences at such low oxygen concentrations would depend upon the sensitivity of the instrument making the oxygen measurement. The dissolved oxygen probe, for example, can measure oxygen concentrations as low as 6.25 x 10^{-6} M (0.2 ppm).* If this instrument was used to measure oxygen concentrations, it would provide identical oxygen traces for the two systems. The reason for this can be seen by comparing the oxygen concentration histories. Down to a concentration of 6.25 x 10^{-6} M, the two systems have almost identical oxygen concentrations (see Tables 27 and 45). In addition, the differences in oxygen concentration

*The sensitivity of the dissolved oxygen probe, in this case the YSI model 15155, is 0.2 ppm.

that do exist are so small ($\sim 1 \times 10^{-7}$ M) that they would not be measured by the dissolved oxygen probe. Hence, the systems would have identical O_2 traces.

The situation described above holds true for the other reaction systems which have different initial conditions. That is, the computer-generated oxygen concentration data indicates large differences in oxygen concentrations between a catalase-containing and a catalase-free system. However, when consideration is given to the type of oxygen monitoring device used (in this case, the dissolved oxygen probe), it becomes apparent that the differences in oxygen concentration would go undetected; identical oxygen traces would be obtained for both the catalase-containing and catalase-free systems.*

The data of Tables 13 through 39 are plotted in the form of oxygen concentration versus time (see Figures 2-10, pages 41-49). In general, the graphs indicate that for a fixed glucose oxidase concentration, the rate of oxygen consumption increases when the initial concentration of glucose (S_o) is increased. Also, for systems with different S_o, the differences in oxygen consumption rate become more apparent with increasing initial oxygen concentration.

The dependence of the rate of oxygen consumption on the glucose and oxygen concentrations is expected. Examination of the rate expression for oxygen consumption (see equation 6) indicates

*Put another way, the quantity of catalase (in the Sigma glucose oxidase preparation) is so low that it does not adversely affect the measurement of oxygen via the dissolved oxygen probe.

that whenever either of these variables is increased (and the other held constant), an increase in oxygen consumption will result.

The data from Tables 40 through 48 are plotted in the form of oxygen concentration versus time (see Figures 11-13). These graphs represent the oxygen traces for catalase-free systems at a variety of initial conditions. As indicated by the graphs, the oxygen traces for catalase-free and catalase-containing systems are identical providing the initial reactant conditions are the same.

FIGURE 2. Oxygen Concentration Curve For The Oxidation of Glucose Via Glucose Oxidase

GLUCOSE OXIDASE PREP.(ML) = .1 PH = 5.5
INITIAL GLUCOSE (M) = .0100
INITIAL OXYGEN (M) T = 30 C

□ .00025
▲ .00125 Glucose Oxidase Activity = .917 units/ml
X .00250 Catalase Activity = 1.23 x 10^{-3} units/ml

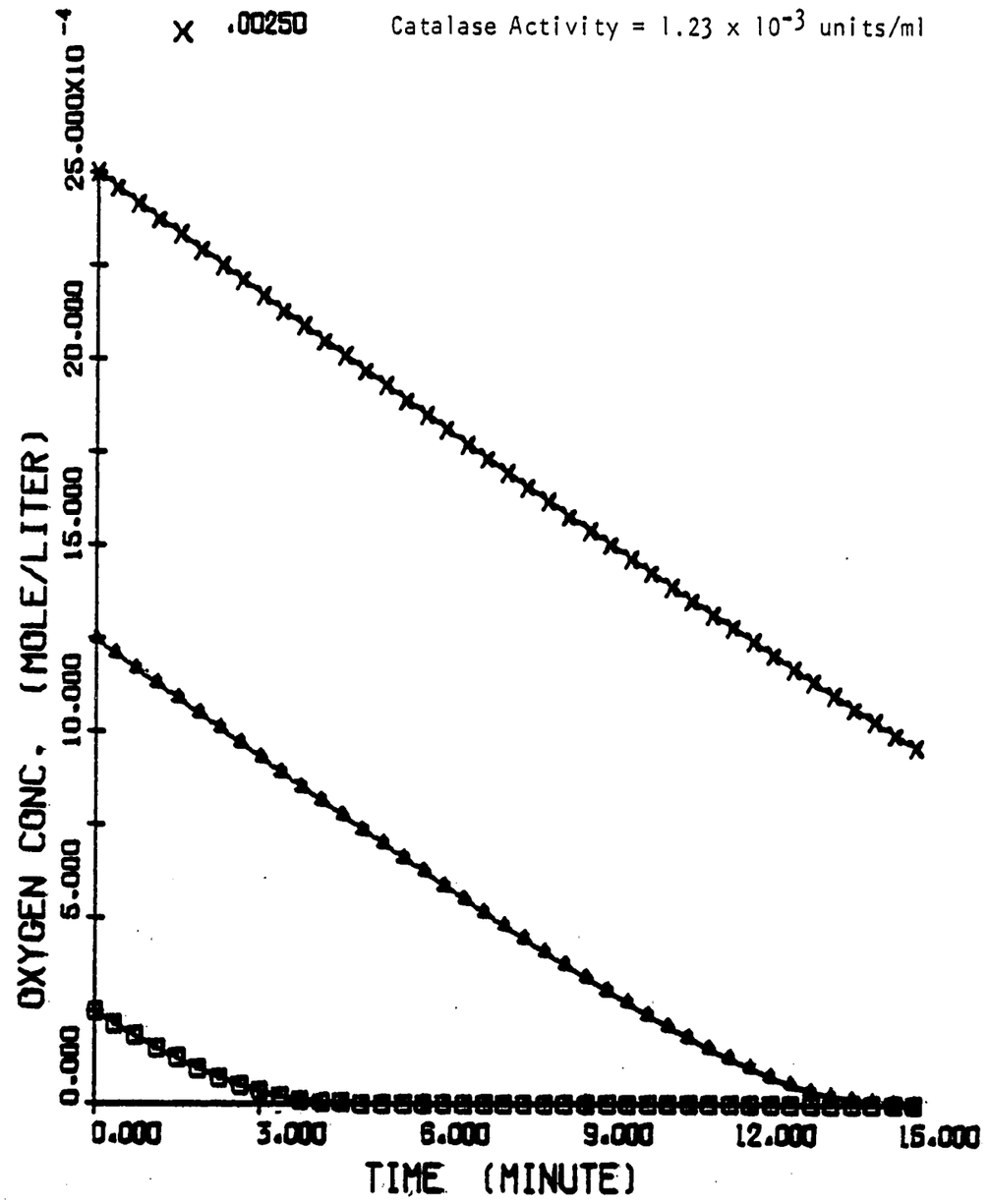


FIGURE 3. Oxygen Concentration Curve For The Oxidation of Glucose Via Glucose Oxidase

GLUCOSE OXIDASE PREP.(ML) = .1 PH = 5.5
INITIAL GLUCOSE (M) = .1000
INITIAL OXYGEN (M) T = 30 C

□ .00025
▲ .00125 Glucose Oxidase Activity = .917 units/ml
× .00250 Catalase Activity = 1.23 x 10^{-3} units/ml

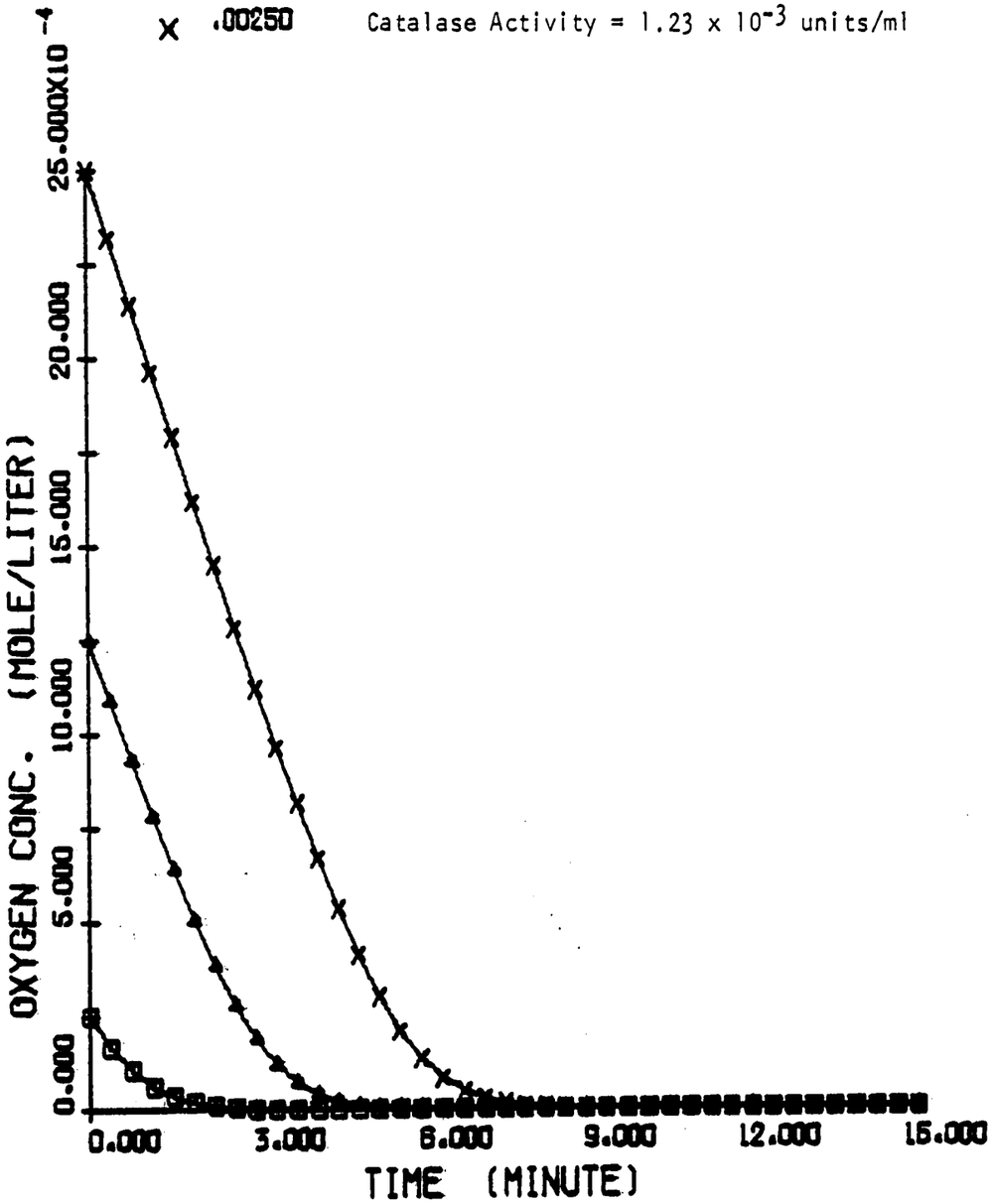


FIGURE 4. Oxygen Concentration Curve For The Oxidation of Glucose Via Glucose Oxidase

GLUCOSE OXIDASE PREP.(ML) = .1 PH = 5.5
INITIAL GLUCOSE (M) = 1.0000
INITIAL OXYGEN (M) T = 30 C

□ .00025
▲ .00125 Glucose Oxidase Activity = .917 units/ml
X .00250 Catalase Activity = 1.23×10^{-3} units/ml

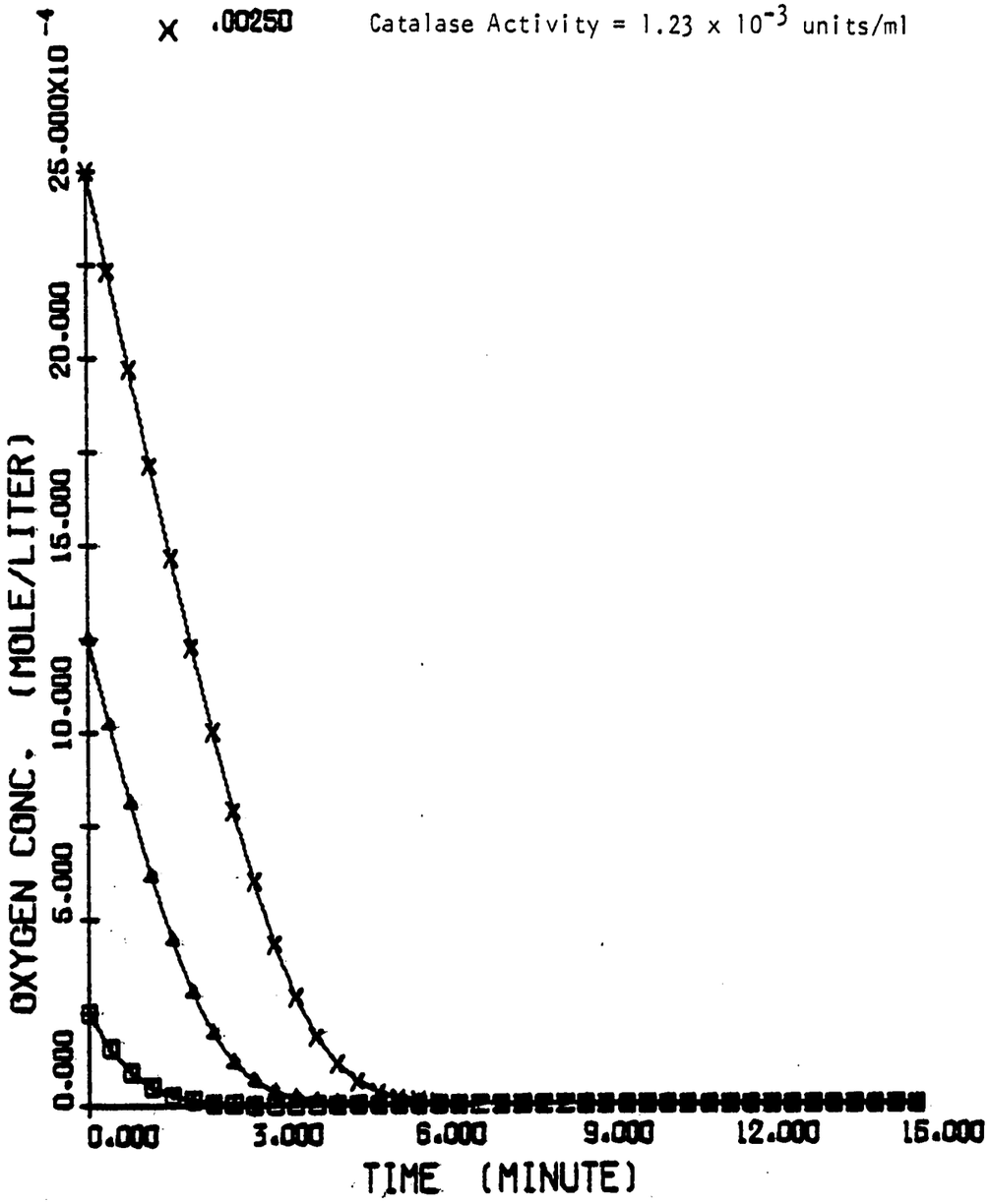


FIGURE 5. Oxygen Concentration Curve For The Oxidation of Glucose Via Glucose Oxidase

GLUCOSE OXIDASE PREP.(ML) = 1.0 PH = 5.5
INITIAL GLUCOSE (M) = .0100
INITIAL OXYGEN (M) T = 30 C

□ .00025
▲ .00125 Glucose Oxidase Activity = 9.17 units/ml
× .00250 Catalase Activity = 1.23 x 10^{-2} units/ml

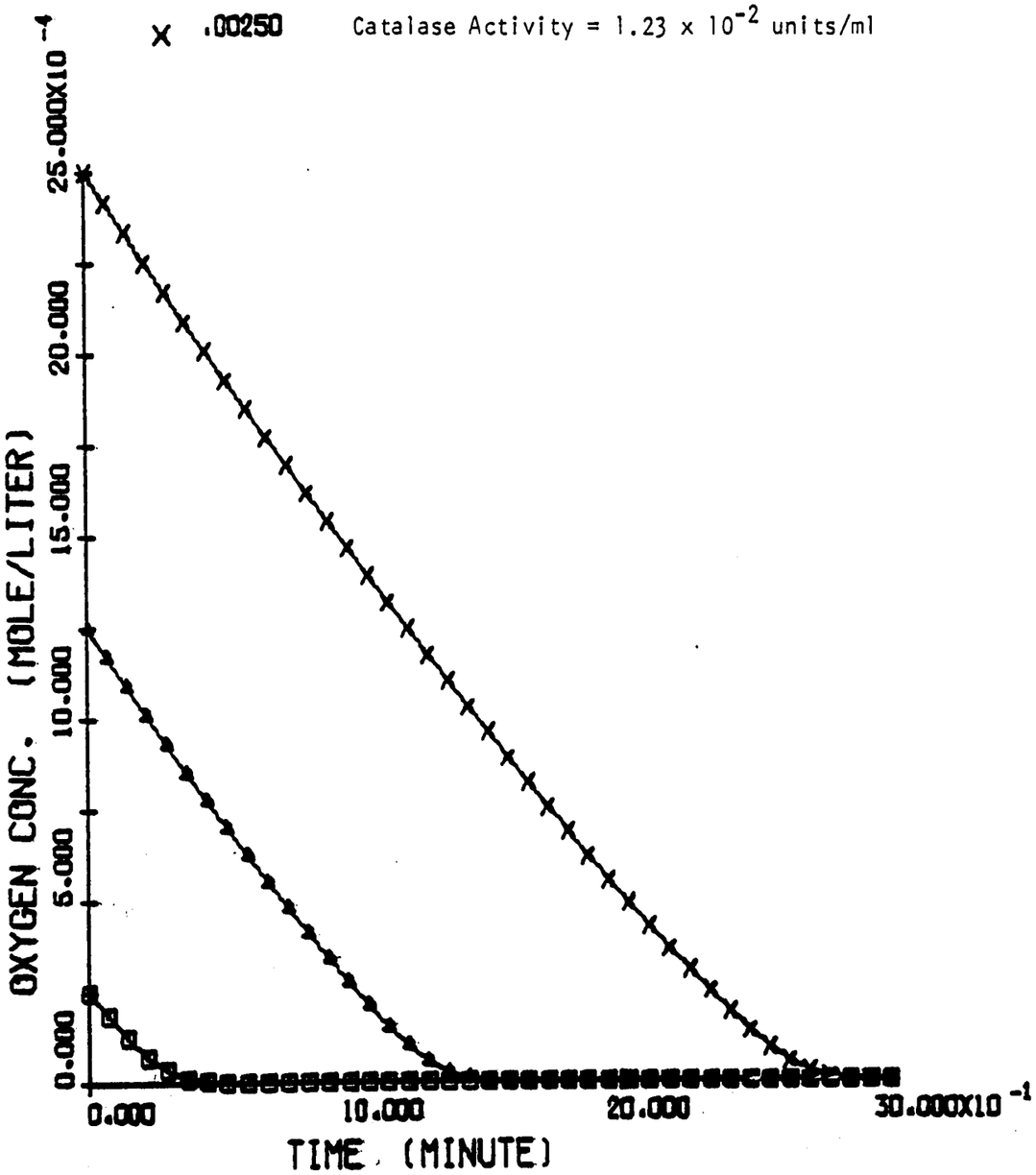


FIGURE 6. Oxygen Concentration Curve For The Oxidation of Glucose Via Glucose Oxidase

GLUCOSE OXIDASE PREP.(ML) = 1.0 PH = 5.5
INITIAL GLUCOSE (M) = .1000
INITIAL OXYGEN (M) T = 30 C

□ .00025
▲ .00125 Glucose Oxidase Activity = 9.17 units/ml
X .00250 Catalase Activity = 1.23 x 10^{-2} units/ml

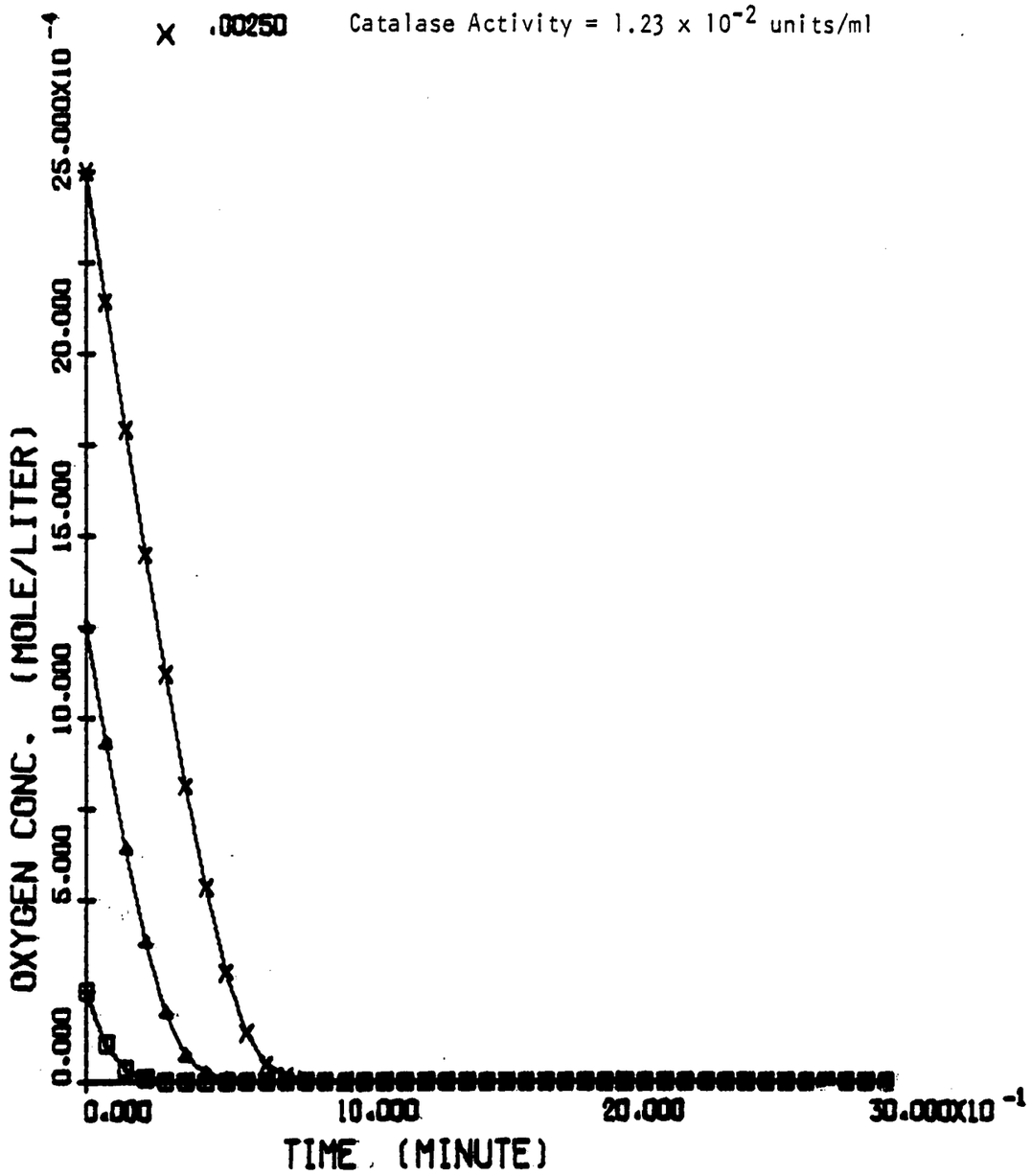


FIGURE 7. Oxygen Concentration Curve For The Oxidation of Glucose Via Glucose Oxidase

GLUCOSE OXIDASE PREP.(ML) = 1.0 PH = 5.5
INITIAL GLUCOSE (M) = 1.0000
INITIAL OXYGEN (M) T = 30 C

□ .00025
▲ .00125 Glucose Oxidase Activity = 9.17 units/ml
X .00250 Catalase Activity = 1.23×10^{-2} units/ml

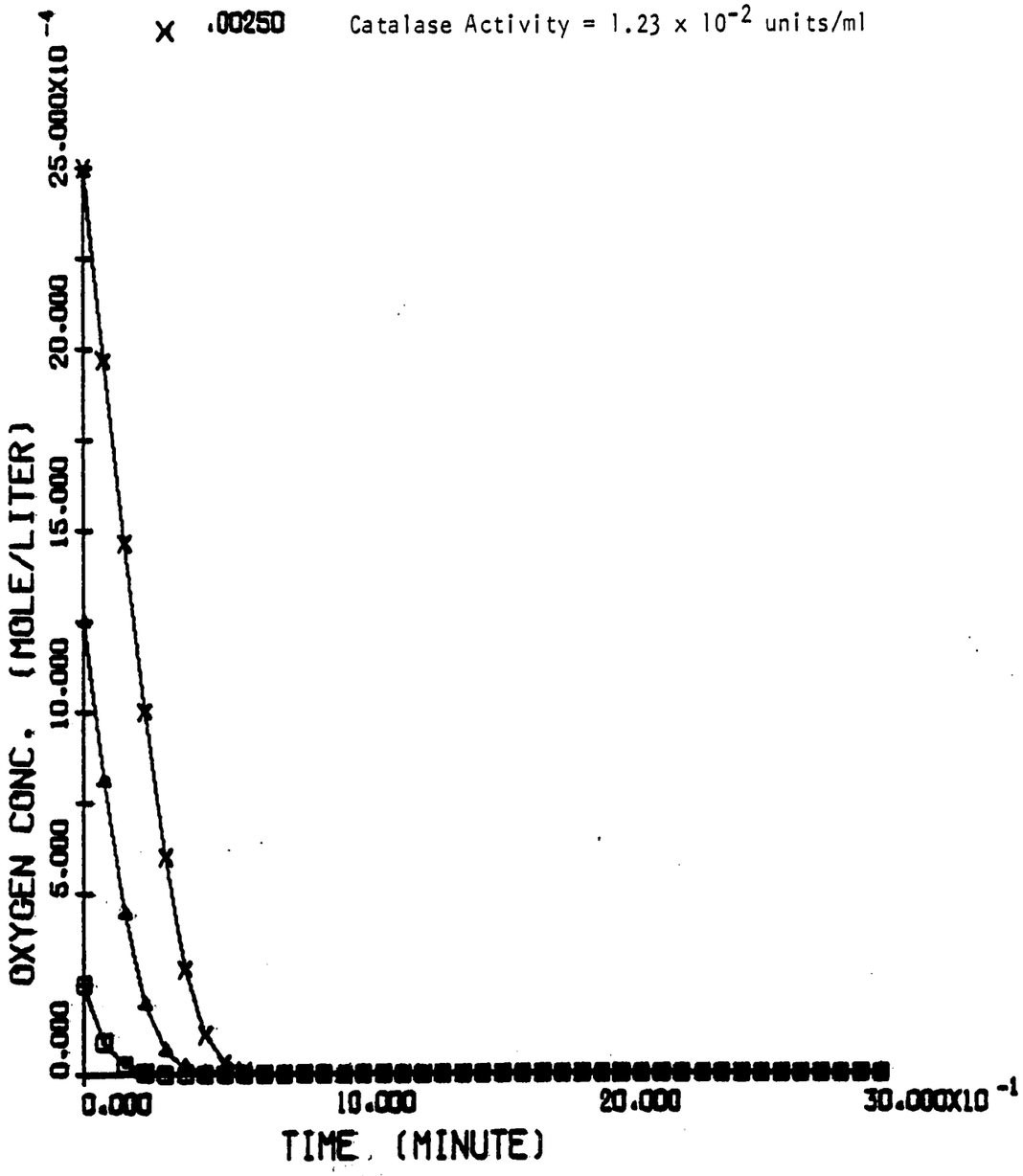


FIGURE 8. Oxygen Concentration Curve For The Oxidation of Glucose Via Glucose Oxidase

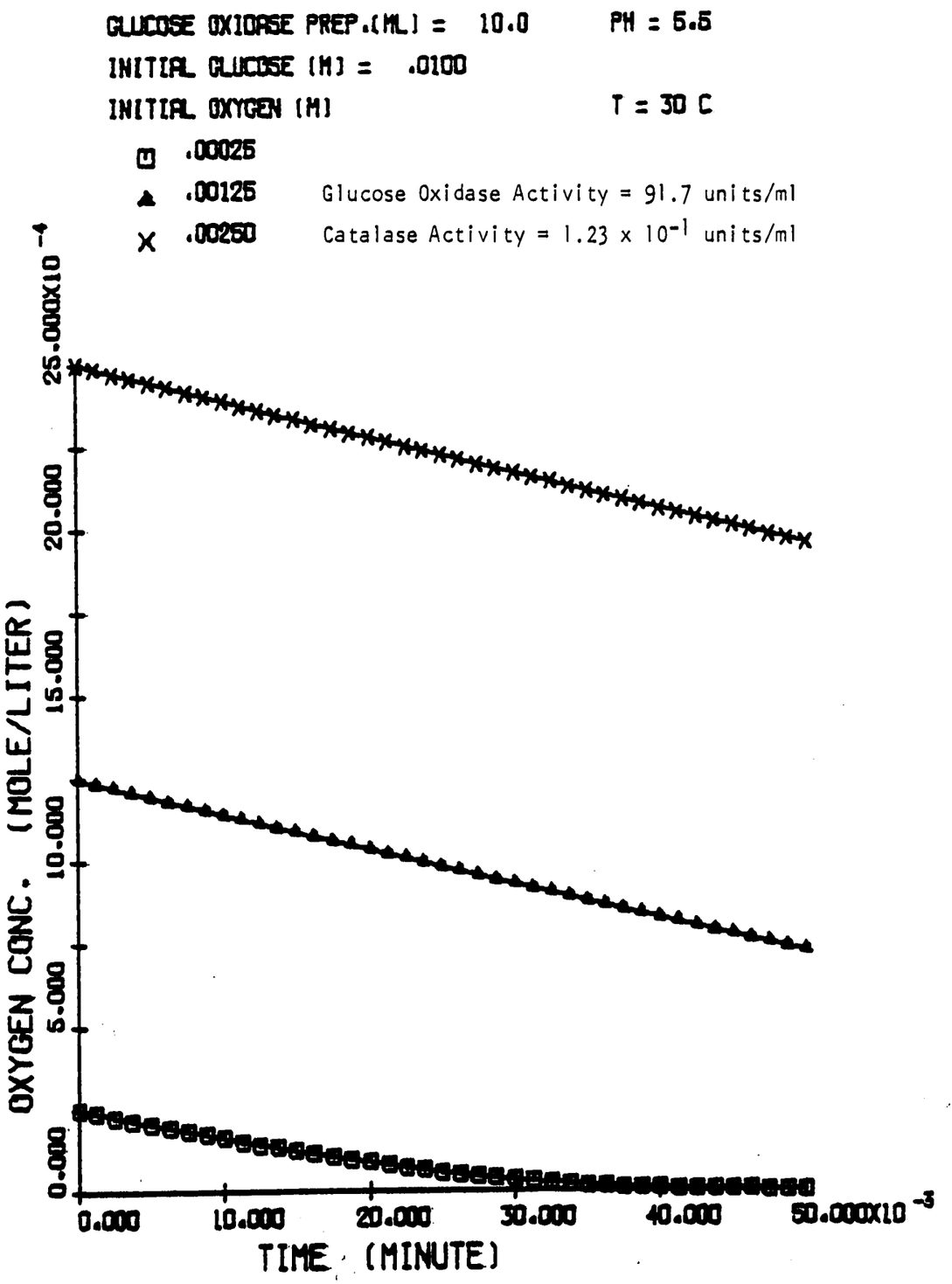


FIGURE 9. Oxygen Concentration Curve For The Oxidation of Glucose Via Glucose Oxidase

GLUCOSE OXIDASE PREP.(ML) = 10.0 PH = 6.6
INITIAL GLUCOSE (M) = .1000
INITIAL OXYGEN (M) T = 30 C

□ .00025
▲ .00125
X .00250

Glucose Oxidase Activity = 91.7 units/ml
Catalase Activity = 1.23×10^{-1} units/ml

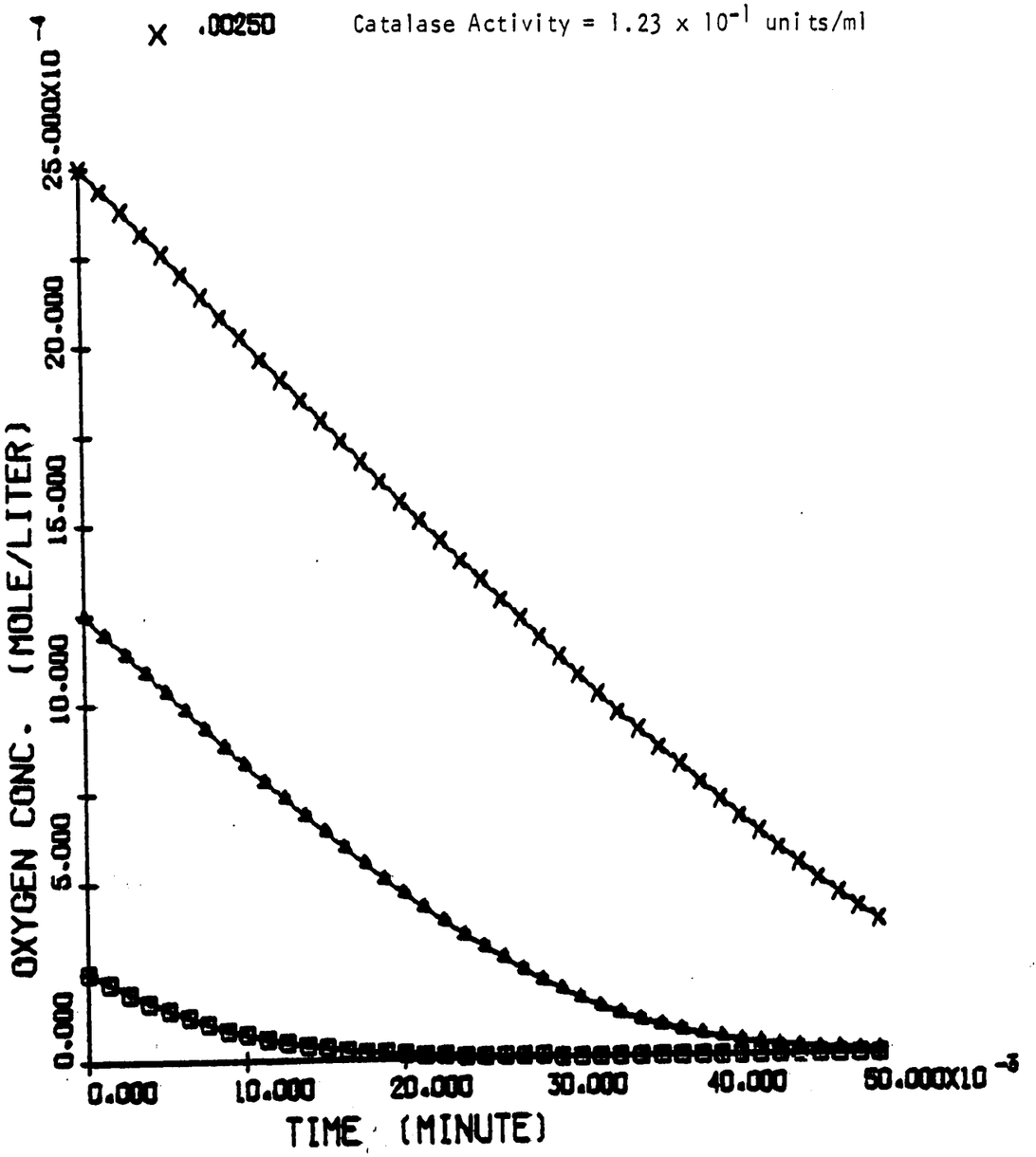


FIGURE 10. Oxygen Concentration Curve For The Oxidation of Glucose Via Glucose Oxidase

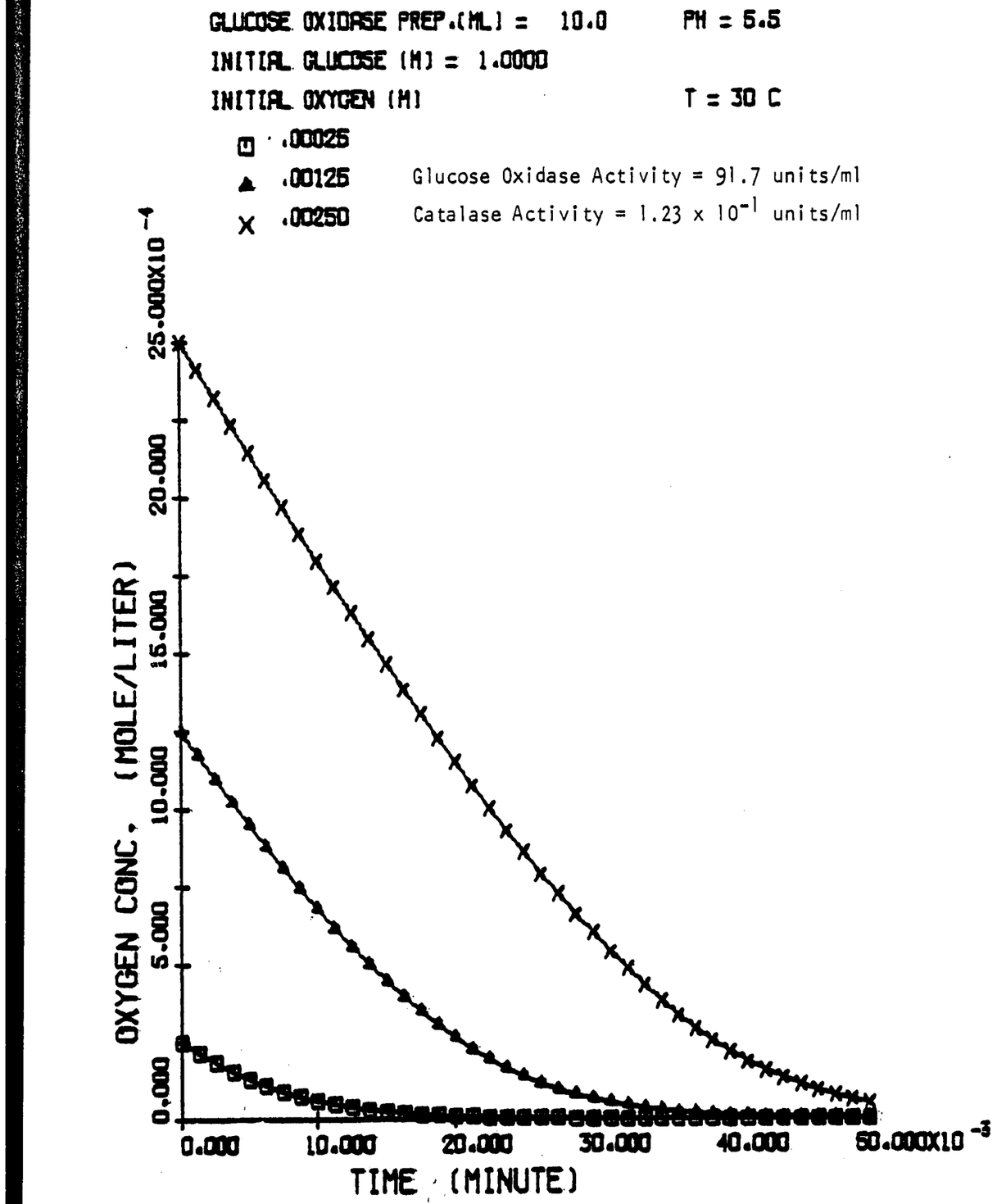


FIGURE 11. Oxygen Concentration Curve For The Oxidation of Glucose Via Glucose Oxidase

GLUCOSE OXIDASE PREP.(ML) = .1 PH = 5.5
INITIAL GLUCOSE (M) = .1000
INITIAL OXYGEN (M) T = 30 C

□ .00025
▲ .00125 Glucose Oxidase Activity = .917 units/ml
× .00250 Catalase Activity = 0.0 units/ml

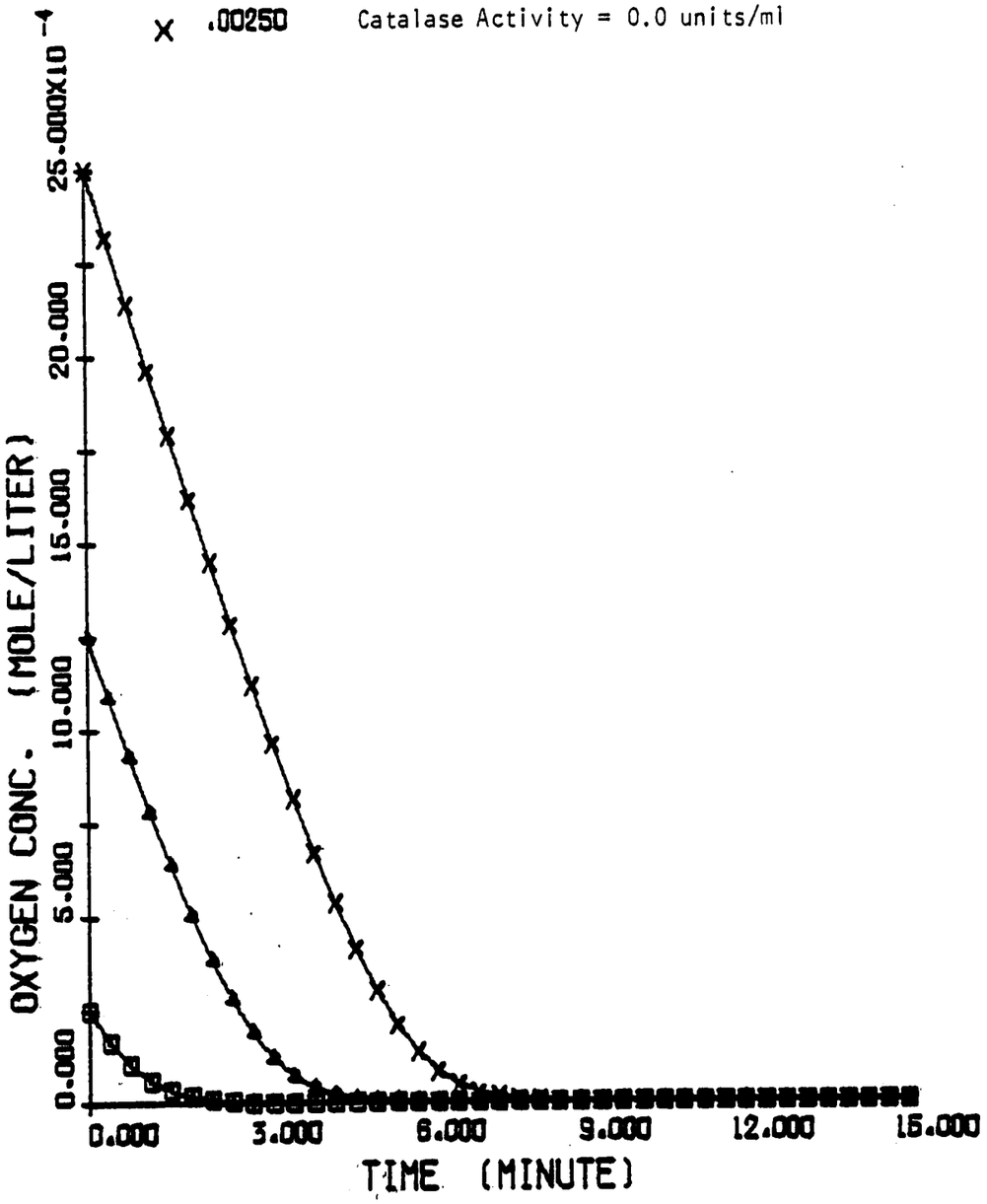


FIGURE 12. Oxygen Concentration Curve For The Oxidation of Glucose Via Glucose Oxidase

GLUCOSE OXIDASE PREP.(ML) = 1.0 PH = 5.5
INITIAL GLUCOSE (M) = .1000
INITIAL OXYGEN (M) T = 30 C

□ .00025
▲ .00125 Glucose Oxidase Activity = 9.17 units/ml
X .00250 Catalase Activity = 0.0 units/ml

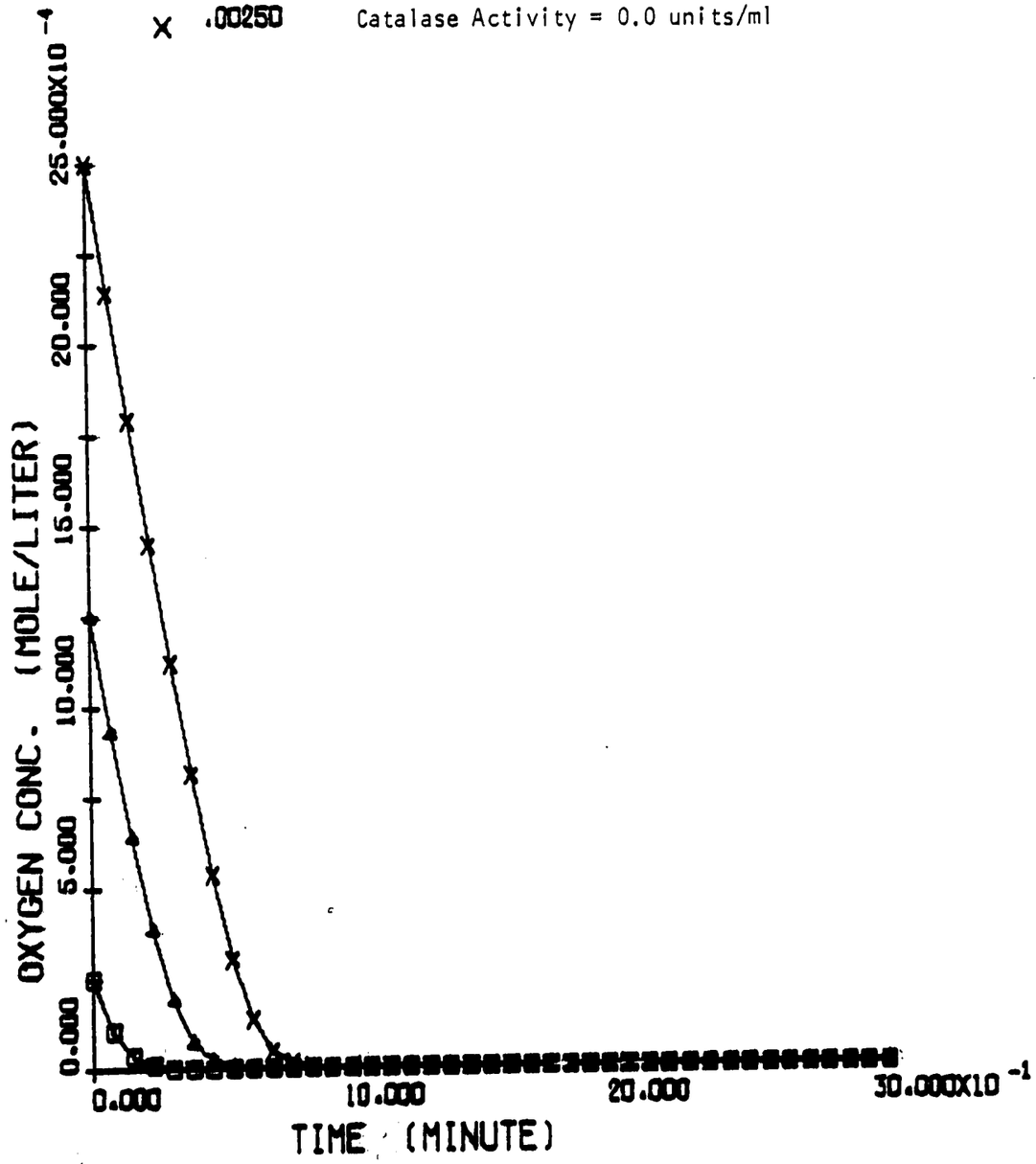
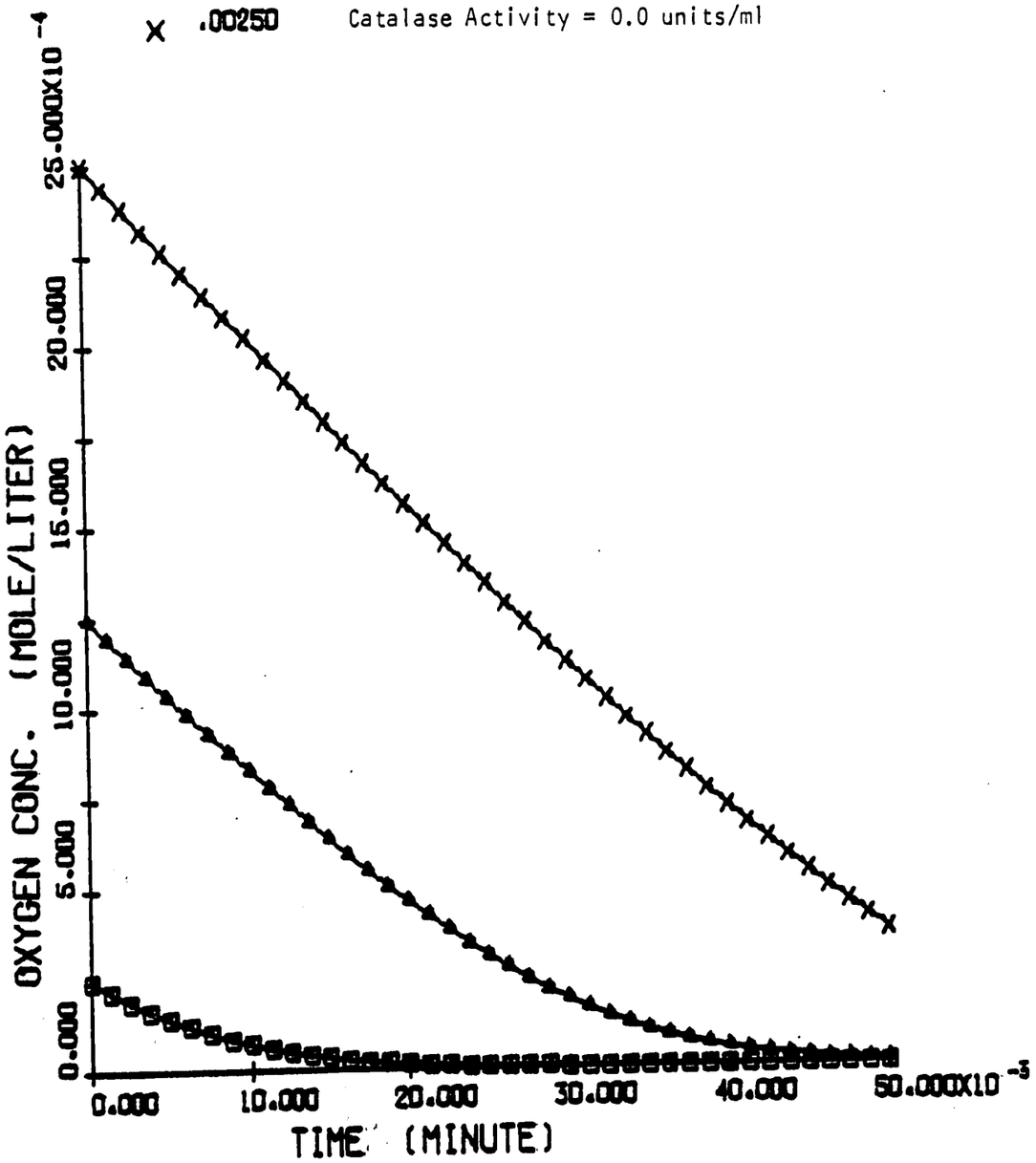


FIGURE 13. Oxygen Concentration Curve For The Oxidation of Glucose Via Glucose Oxidase

GLUCOSE OXIDASE PREP.(ML) = 10.0 PH = 5.5
INITIAL GLUCOSE (M) = .1000
INITIAL OXYGEN (M) T = 30 C

□ .00025
▲ .00125 Glucose Oxidase Activity = 91.7 units/ml
X .00250 Catalase Activity = 0.0 units/ml



4.3.3 The Conversion of Gluconolactone to Gluconic Acid

The concentration data for gluconolactone and gluconic acid are listed in Tables 13 to 48, pages 101-136. The data indicates that, regardless of the initial reactant conditions, the conversion of gluconolactone to gluconic acid occurs at a slow rate: it can be concluded that the Sigma glucose oxidase preparation is deficient in lactonase.

The percent conversion of gluconolactone to gluconic acid has been calculated for the various systems at the termination of the reaction.* The data, listed in Tables 51 to 53, pages 149-151 , indicates an apparent contradiction--the highest conversions occurred in systems which contained the least amount of glucose oxidase. Actually, the higher conversions were due to the longer reaction times associated with the lower concentrations of enzyme preparation. The longer reaction time allowed for more of the lactone to hydrolyze spontaneously.

The data of Tables 51 to 53 further supports the conclusion that the Sigma glucose oxidase preparation was deficient in lactonase.

Table 54 (page 152) compares lactone conversions for systems with identical reaction times and initial reactant concentrations, but which have different glucose oxidase activities. As expected, systems with greater concentrations of glucose oxidase preparation had higher lactone conversions.

*The reaction time varied according to the activity of glucose oxidase present in the system. For glucose oxidase activities of .917 units/ml, 9.17 units/ml, and 91.7 units/ml, the reaction times were 15 minutes, 3 minutes, and 3 seconds, respectively.

The gluconic acid concentration data of Tables 13 through 39 is plotted as a function of time (see Figures 14-22, pages 56-64). From these graphs several observations can be made. First, for fixed quantities of oxygen and enzyme preparation, the rate of gluconic acid production increases with increasing (initial) concentrations of glucose. (For example, when X = 10.0 ml and $O_{2o} = 2.5 \times 10^{-3}$ M, the rate of gluconic acid production is greater when S_o = 1.0 M than when S_o = 0.1 M). Second, for systems with identical glucose oxidase activity, the rates of gluconic acid production are more dependent on oxygen concentration as the glucose concentration is increased; the differences in gluconic acid production, among the various initial oxygen concentrations, become more apparent at higher glucose concentrations. Third, more gluconic acid is produced when the initial concentration of either oxygen or glucose is increased.

The increase in gluconic acid production with increasing (initial) glucose concentration is expected. The expression (see equation 8) for the rate of lactone production shows that increases in glucose concentration will result in increased lactone concentrations which, in turn, will cause the formation of more gluconic acid. A similar situation exists for oxygen; higher oxygen concentrations result in greater lactone production and, therefore, an increase in gluconic acid production.

4.3.4 The Gluconic Acid Concentration Curves For a Catalase-Free System

Figures 23 through 25, pages 65-67, represent the gluconic acid concentration curves for a catalase-free system with an initial

glucose concentration of 0.1 M. The curves are identical to those of a catalase-containing system which has the same initial conditions.

FIGURE 14. Gluconic Acid Concentration Curve For The Glucose Oxidase Reaction System

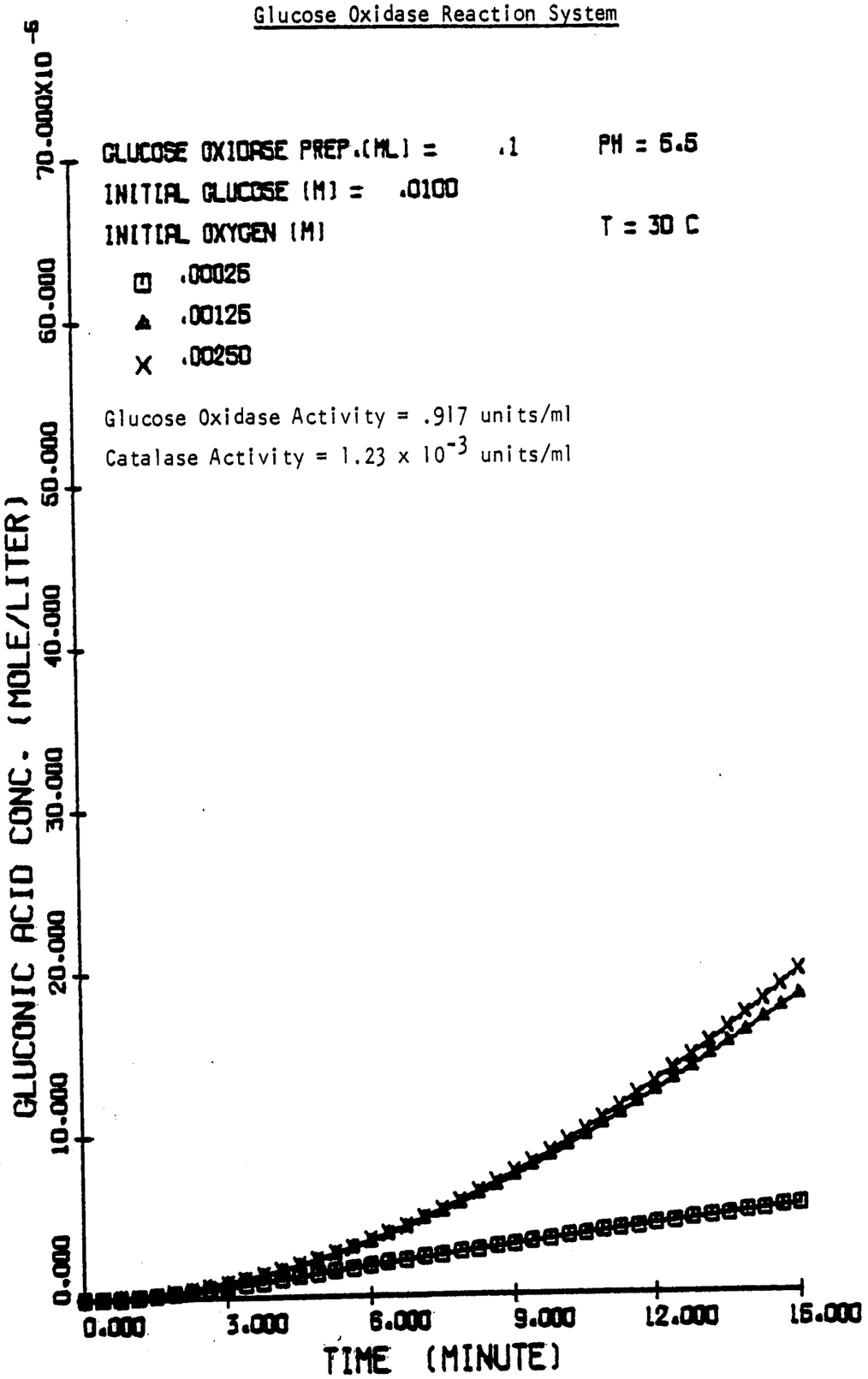


FIGURE 15. Gluconic Acid Concentration Curve For The Glucose Oxidase Reaction System

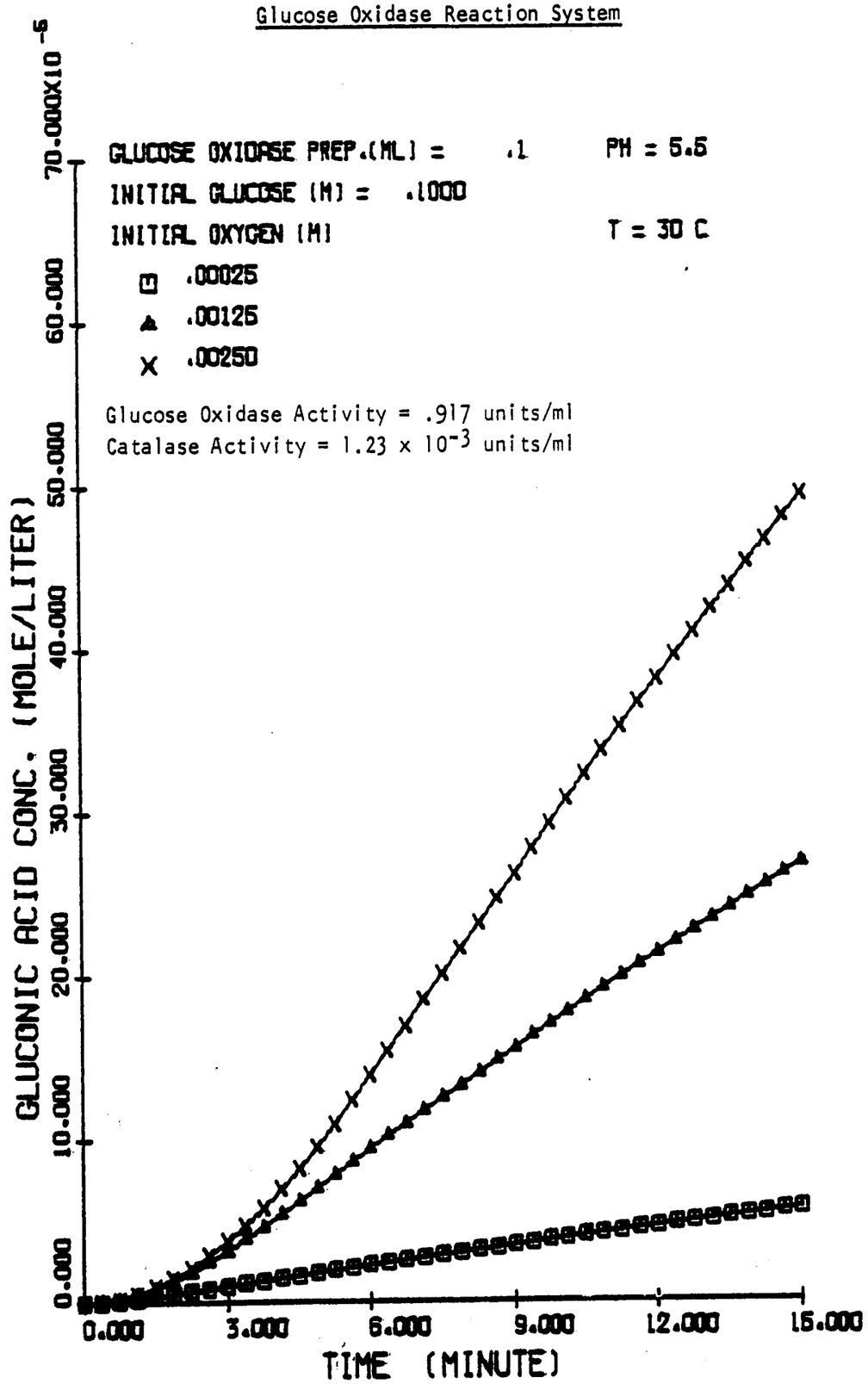


FIGURE 16. Gluconic Acid Concentration Curve For The Glucose Oxidase Reaction System

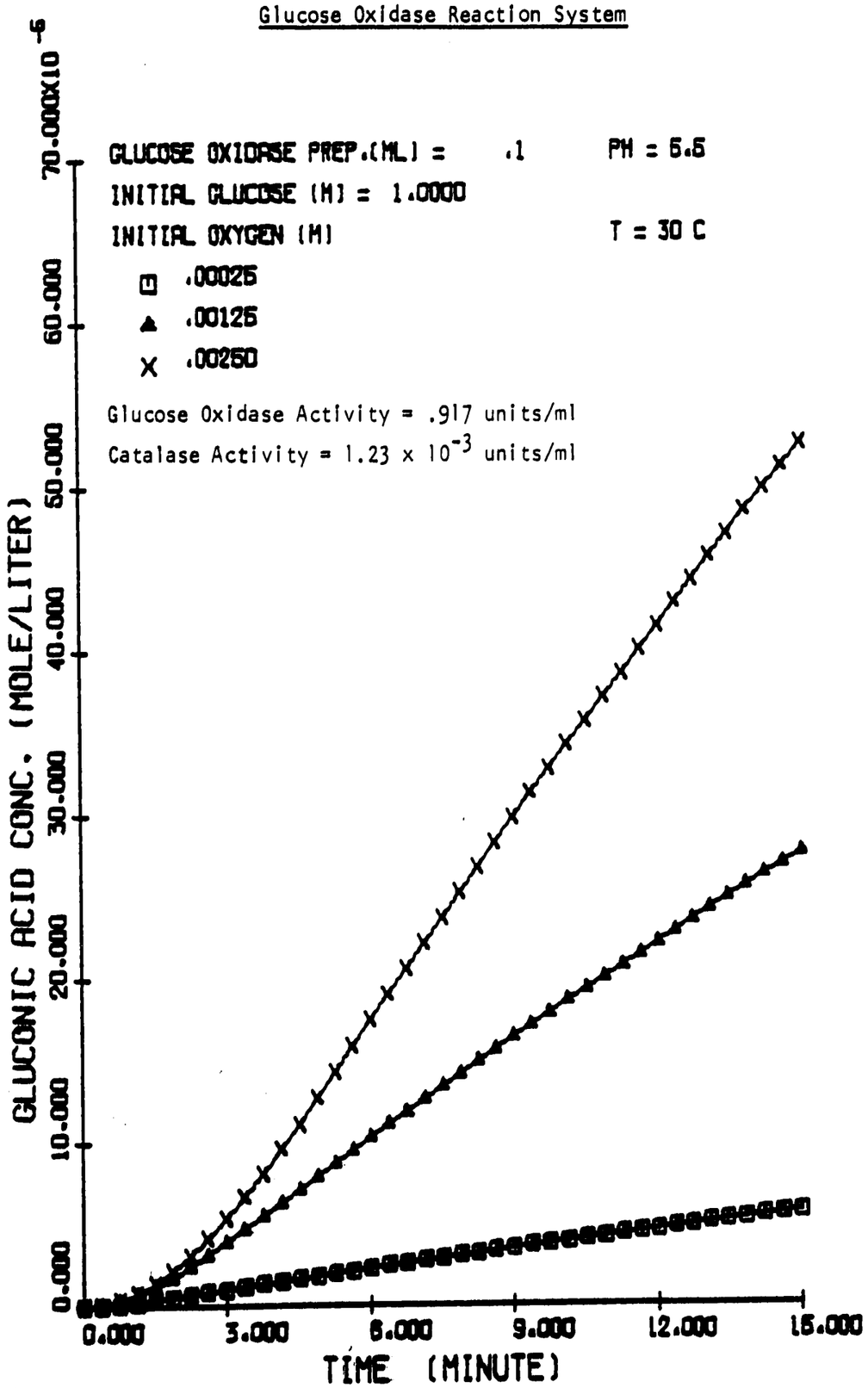


FIGURE 17. Gluconic Acid Concentration Curve For The Glucose Oxidase Reaction System

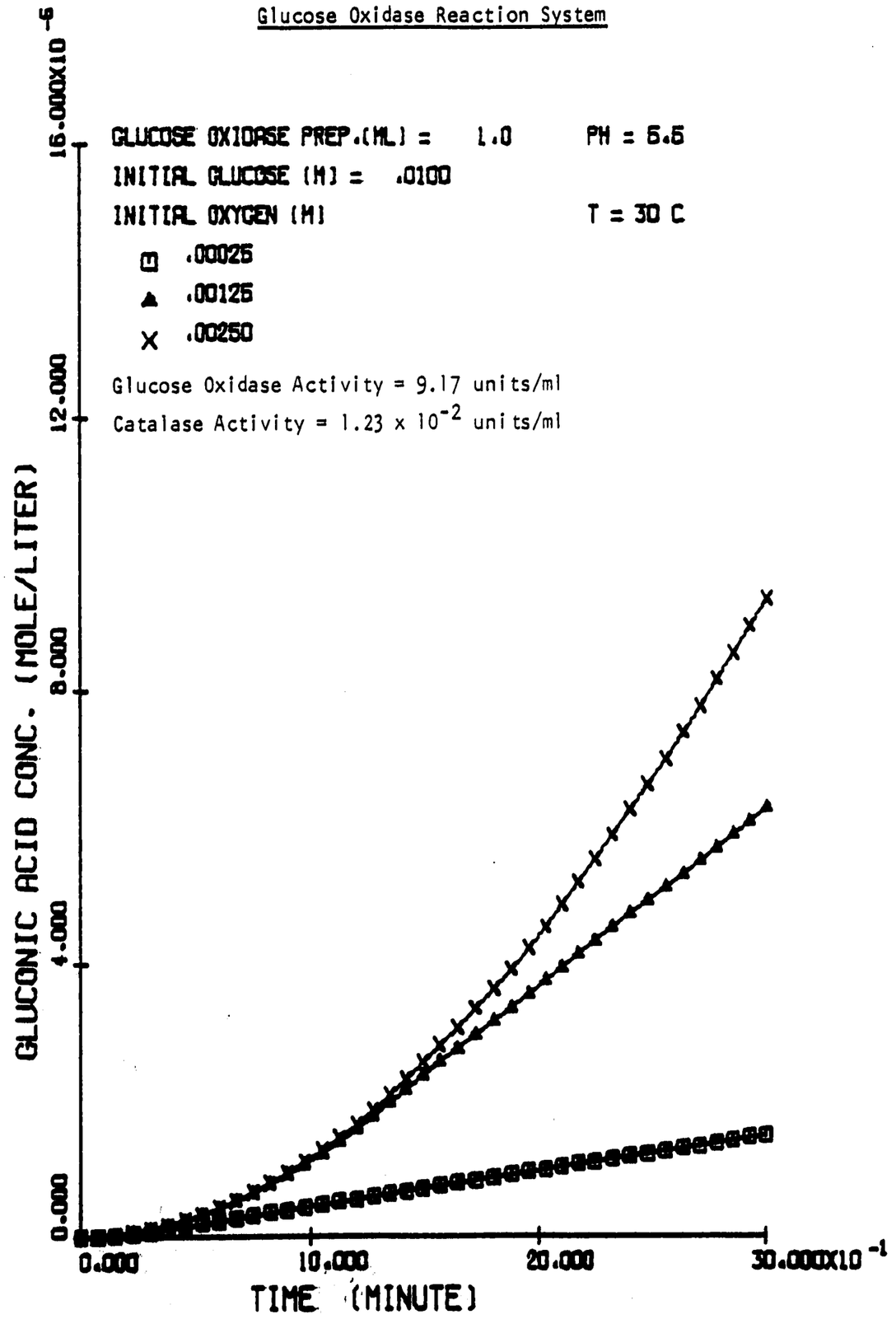


FIGURE 18. Gluconic Acid Concentration Curve For The Glucose Oxidase Reaction System

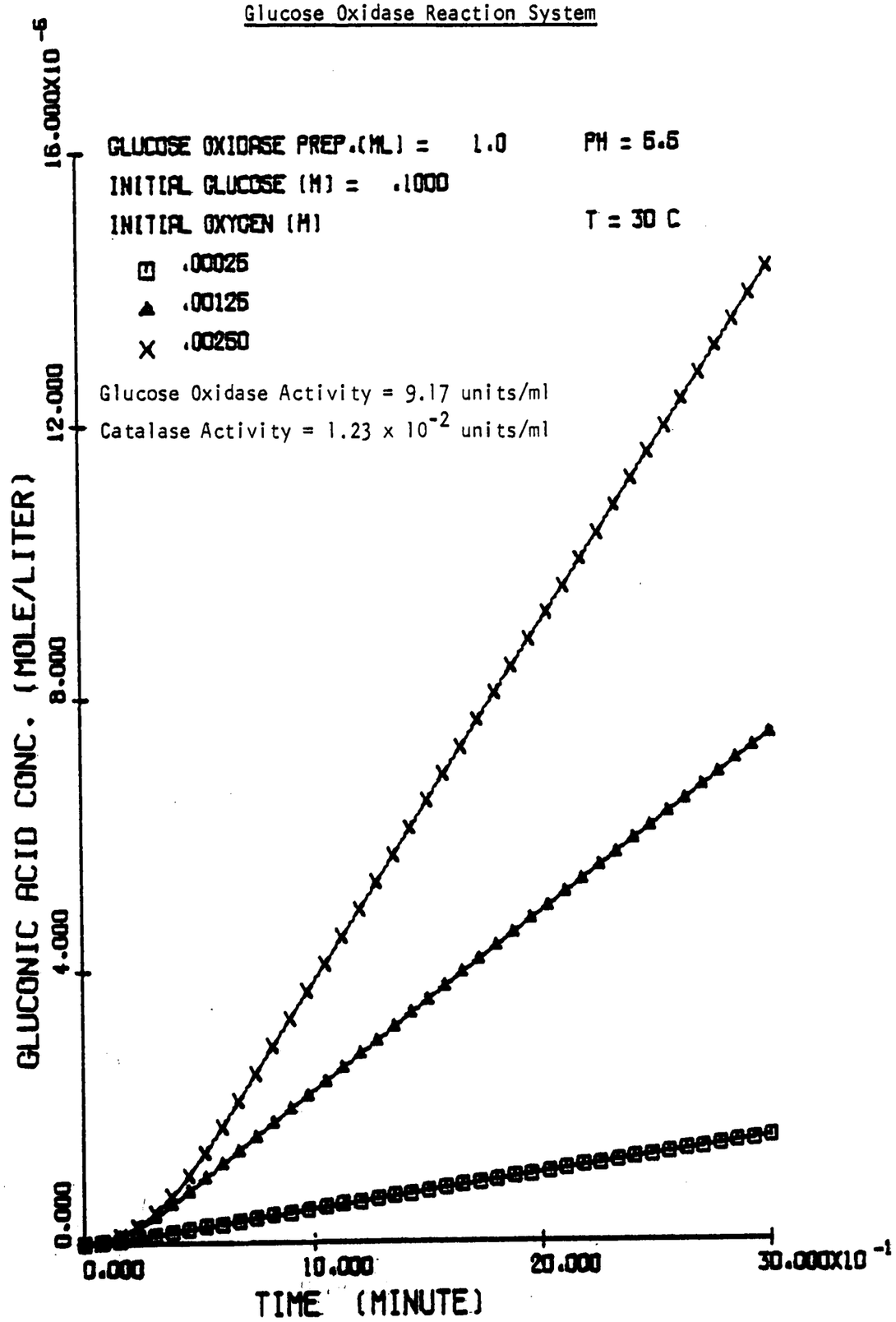


FIGURE 19. Gluconic Acid Concentration Curve For The Glucose Oxidase Reaction System

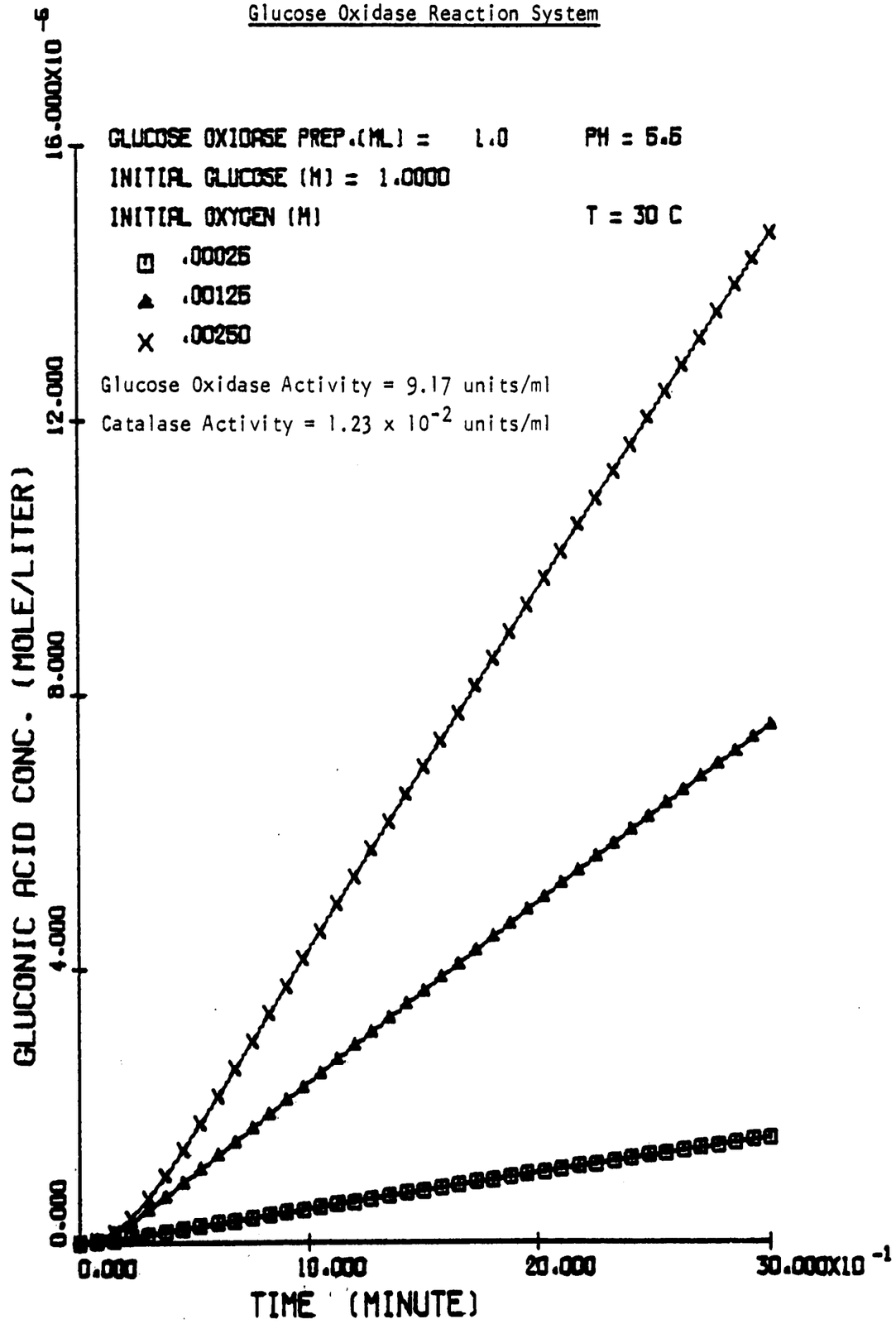


FIGURE 20. Gluconic Acid Concentration Curve For The Glucose Oxidase Reaction System

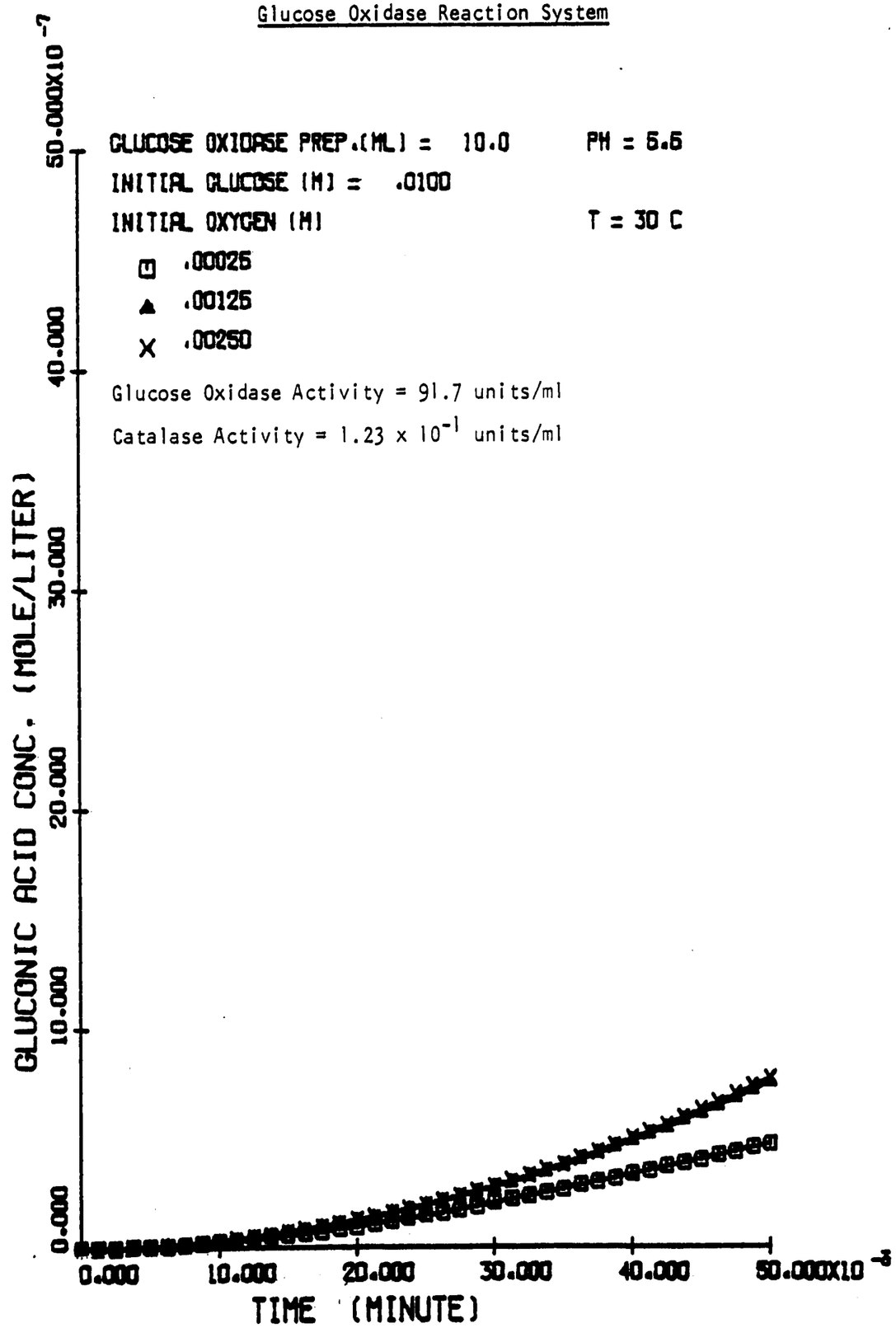


FIGURE 21. Gluconic Acid Concentration Curve For The Glucose Oxidase Reaction System

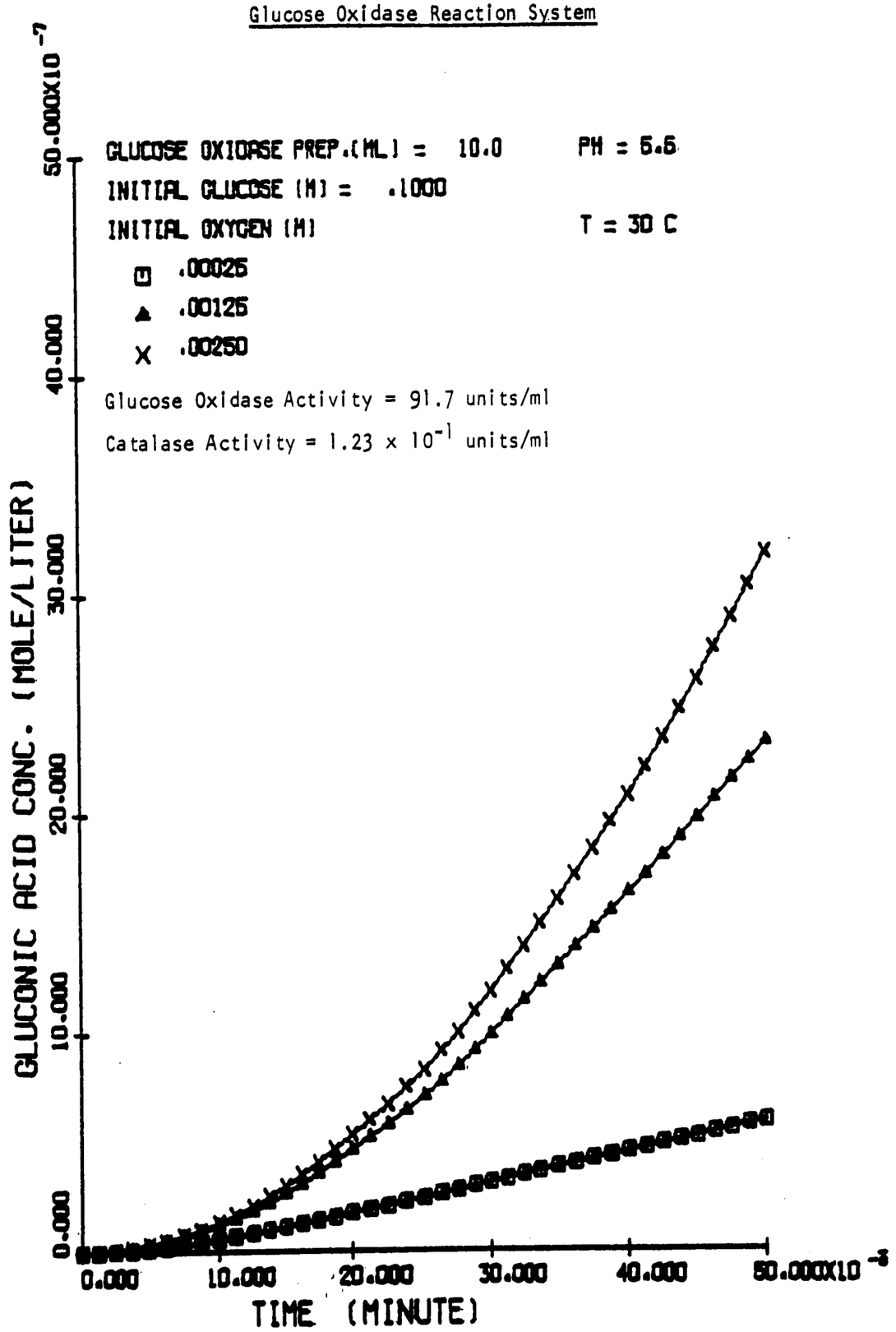


FIGURE 22. Gluconic Acid Concentration Curve For The Glucose Oxidase Reaction System

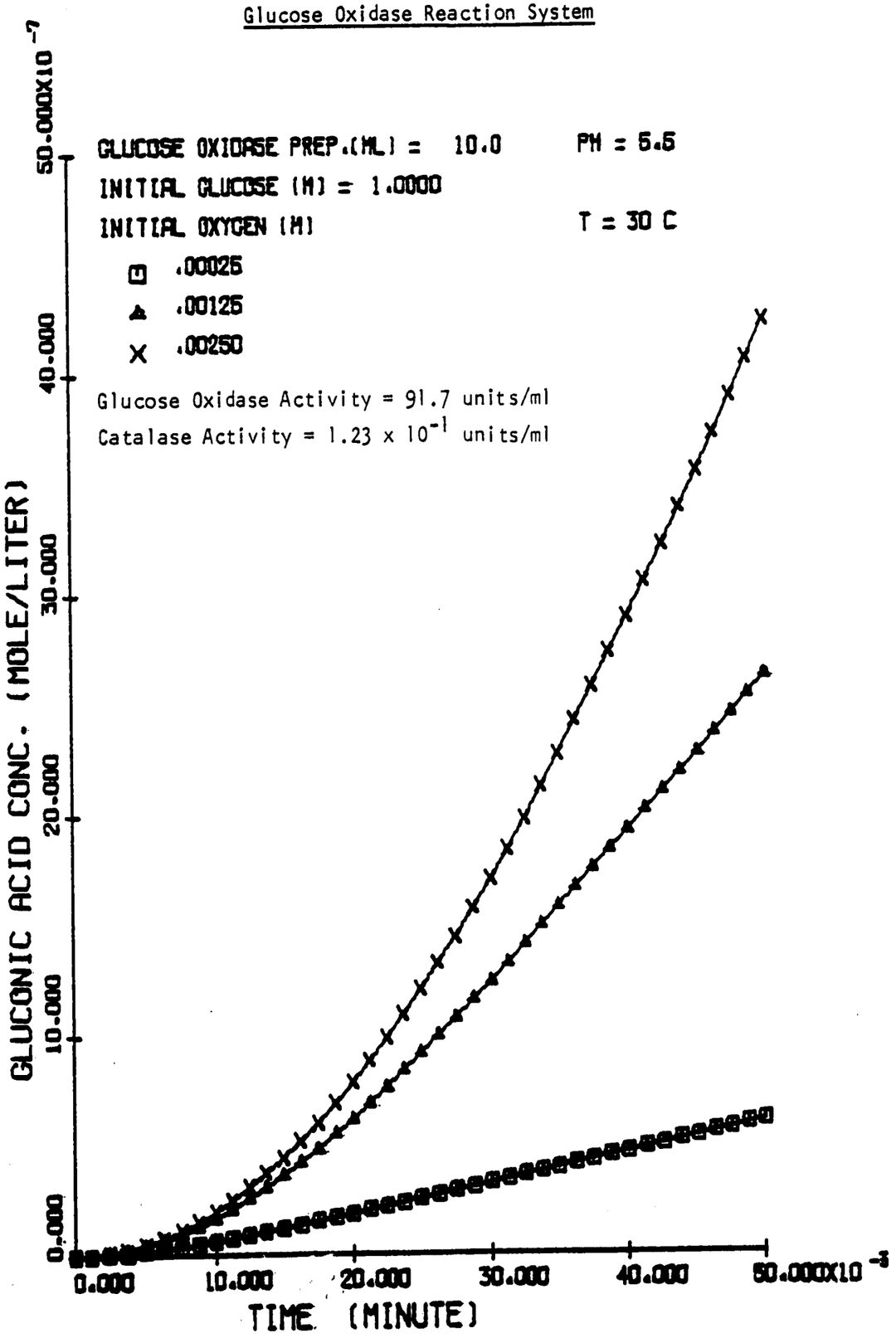


FIGURE 23. Gluconic Acid Concentration Curve For The Glucose Oxidase Reaction System

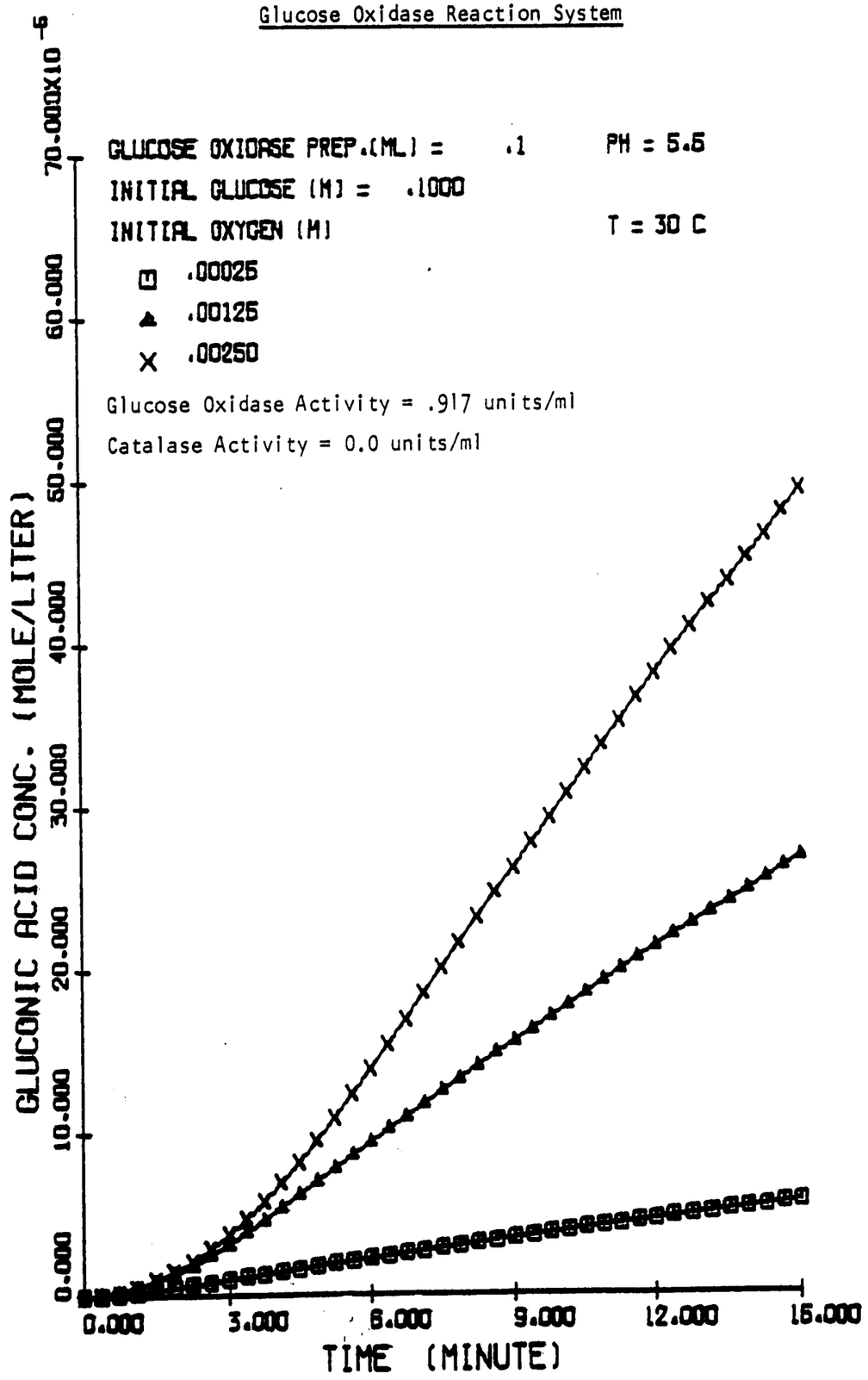


FIGURE 24. Gluconic Acid Concentration Curve For The Glucose Oxidase Reaction System

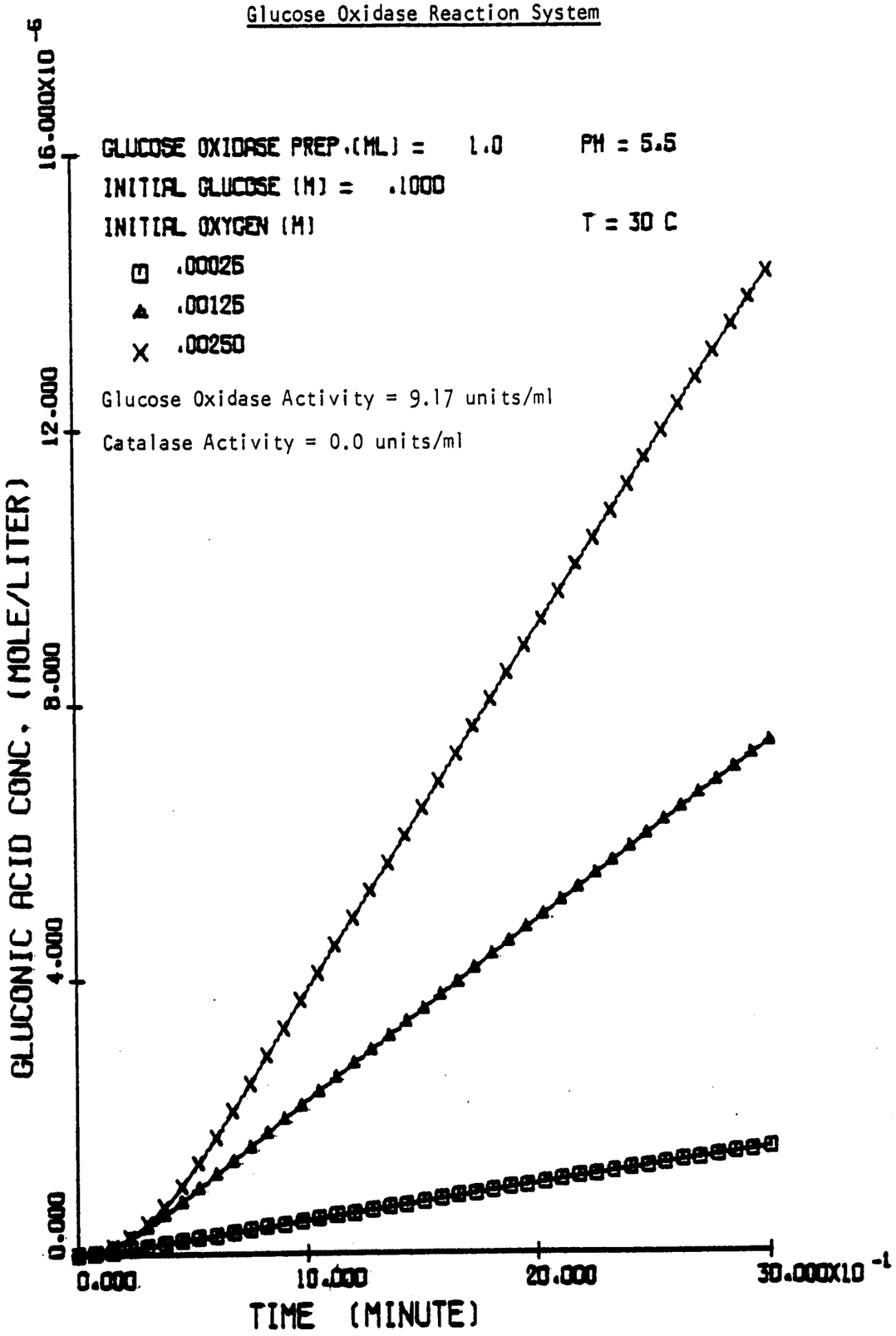
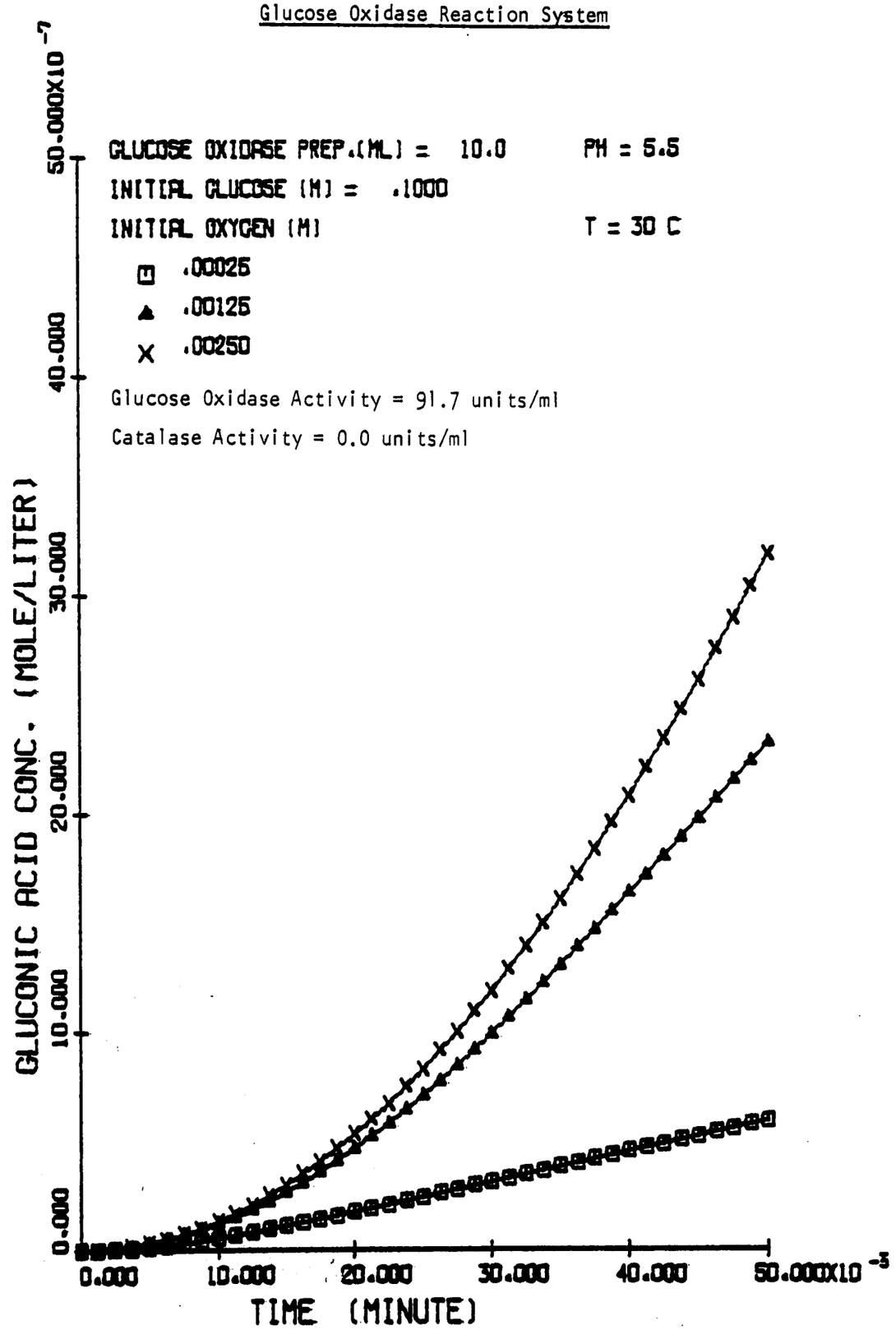


FIGURE 25. Gluconic Acid Concentration Curve For The Glucose Oxidase Reaction System



4.4 Determining The Effect of Gluconic Acid, Gluconolactone, and Hydrogen Peroxide on The YSI Glucose Analyzer

4.4.1 The Effect of Gluconic Acid on The YSI Glucose Analyzer

Table 9, page 91 lists the data obtained when various solutions of gluconic acid were analyzed using the glucose analyzer. As indicated by the data, the glucose analyzer did not respond to any of the gluconic acid solutions. Therefore, it can be concluded that the presence of gluconic acid will not interfere with glucose measurements made with the glucose analyzer.

4.4.2 The Effect of Gluconolactone on The YSI Glucose Analyzer

Table 9, page 91, lists the data that were obtained when various solutions of glucono-δ-lactone were analyzed via the glucose analyzer. The data shows a response by the instrument which appears to be dependent on the lactone concentrations of the solutions.

The glucose analyzer may have been responding to glucose impurities that were present in the lactone solutions, rather than to the gluconolactone itself. The gluconolactone (obtained from Sigma Chemical Company) used for these tests contained 99.7% gluconolactone; the remaining 0.3% consisted of various impurities, including glucose.* If glucose accounted for all of the 0.3% impurities, then for a 1.0 M solution of lactone, a glucose concentration of 54 mg/dl would exist. This concentration is approximately three times that which was registered by the glucose

*The data, concerning the lot analysis of the gluconolactone, was obtained in a private communication with Sigma Chemical Company.

analyzer. Thus, it is possible that the analyzer was measuring glucose impurities rather than gluconolactone. Further tests would most likely verify this conclusion.

4.4.3 The Effect of Hydrogen Peroxide on The YSI Glucose Analyzer

Standardized hydrogen peroxide solutions (see Appendix A, page 71, for methods of standardizing H_2O_2 solutions) were injected into the glucose analyzer and the analyzer's response was recorded. Table 9, page 91, lists the data obtained for a variety of hydrogen peroxide solutions.

The data indicates a very erratic response to the H_2O_2 solutions by the glucose analyzer. The situation is further complicated by the fact that the analyzer's response varies with time; that is, different readings were obtained from consecutive analyses of the same hydrogen peroxide solution. Additional studies are necessary before conclusions can be made regarding the effect that H_2O_2 solutions have on the analyzer's performance.

APPENDICES

APPENDIX A

EXPERIMENTAL PREPARATION AND PROCEDURES

A.1 Preparation and Standardization of 0.1 N $KMnO_4$ Solution

To prepare a 0.1 N solution, 3.2 grams of $KMnO_4$ are dissolved in one liter of distilled water.

A method for standardizing the potassium permanganate solution is given in "Fundamentals of Analytical Chemistry".[24] The procedure is listed below.

> Method of McBride. Dissolve samples of $Na_2C_2O_4$ (weighed to the nearest 0.1 mg) in a solution prepared by diluting 30 ml of 6N H_2SO_4 to about 250 ml. Heat to 80°C to 90°C and titrate with the $KMnO_4$, stirring vigorously with a thermometer. The first addition of reagent should be made slowly enough so that the pink color is discharged before further additions are made. If the temperature falls to 60°C, heat. The endpoint is the first persistent pink color. Correct the titration for an endpoint blank determined by titrating an equal volume of the water and acid.

Notes:

1) Typically, the above procedure had a precision of 0.1% deviation from the mean.

A.2 Standardization of Hydrogen Peroxide Solutions

A method for standardizing hydrogen peroxide solutions is given below:

> Transfer a sample aliquot containing 20 to 60 mg of hydrogen peroxide to a 250 ml Erlenmeyer flask. Dilute to 50 ml with water and add 3 ml of 6M sulfuric acid and two drops of 5% aqueous manganous sulfate solution. Titrate with standardized 0.100 N potassium permanganate solution to the first pink color.

Carry out a blank determination by titrating 3 ml of 6M sulfuric acid diluted with 50 ml of water.

$$H_2O_2,\ wt\% = \frac{(A-B)(N)(17.007)(100)}{(W)(1000)}$$

where: A = volume of permanganate solution used for sample titration, in ml.

B = volume of permangate solution used for blank titration, in ml.

N = normality of the permanganate solution.

W = sample weight in grams (25)

Notes:

1) Using the above procedure, hydrogen peroxide concentrations can be calculated in terms of moles per liter from the following expression:

$$H_2O_2,\ (moles/liter) = \frac{(A-B)(N)(17.007)}{V\ (M.W._{H_2O_2})}$$

where: V = volume of the hydrogen peroxide sample, in ml.

M.W. = molecular weight of hydrogen peroxide (34.01 gm/mole)

A,B,N = see previous definitions used above.

2) The precision that was obtained with the above method was very good; the maximum percent deviation from the mean was .25%.

A.3 Preparation of Buffers For pH = 5.5

Potassium Hydrogen Phthalate/Sodium Hydroxide Buffer

11.79 grams Potassium hydrogen phthalate

1.7 grams Sodium hydroxide

Dissolve reagents in water and q.s. to one liter.

Sodium Phosphate Dibasic/Citric Acid Buffer

29.9 grams $Na_2HPO_4 \cdot 7\ H_2O$

9.3 grams $C_6H_8O_7 \cdot H_2O$

Dissolve reagents in water and q.s. to one liter.

Sodium Acetate/Acetic Acid Buffer

68.0 grams $CH_3COONa \cdot 3\ H_2O$

4.5 ml concentrated acetic acid

Dissolve reagents in water and q.s. to one liter.

A.4 Preparation of Buffers For pH = 7.0

Potassium Dihydrogen Phosphate/Sodium Hydroxide Buffer

8.6 grams KH_2PO_4

1.5 grams NaOH

Dissolve in water and q.s. to one liter.

A.5 Evaluating Buffer Systems For Use in The Gluconolactone Assay

Procedure:

Various buffer systems (potassium hydrogen phthalate-sodium hydroxide; citric acid-sodium phosphate dibasic) were tested to find one that was most suitable for use in the gluconolactone assay (see page 23). The method used for evaluating the buffers is as follows.

Two liters of pH = 5.5 buffer solution were prepared (see page 72 for the preparation of the buffer solutions). One liter of the buffer solution was used to make a 4.36×10^{-2} M gluconolactone "stock" solution. The remaining buffer solution was used to prepare dilutions of the gluconolactone "stock" solution.

The diluted lactone solutions (whose concentrations were known) were subjected to the gluconolactone assay and the absorbance data, for each of the solutions, was recorded.

The absorbance/concentration data for the buffered lactone solutions was examined to see how well it correlated with the gluconolactone calibration curve (see page 84 for the calibration curve). From this analysis, it was determined which buffer solutions could be used for the gluconolactone assay.

Results:

The potassium hydrogen phthalate-sodium hydroxide buffer proved to be unsuitable for the gluconolactone assay; at low pH (pH ≈ 1.0), there was precipitate formation.

The citric acid-sodium phosphate dibasic buffer encountered no problems (precipitate formation) when used in the gluconolactone assay. In addition, its absorbance/concentration data indicated (when compared to the gluconolactone calibration curve) no interference with the assay reagents by the buffer components.

The absorbance/concentration data for gluconolactone solutions prepared in a citric acid-sodium phosphate dibasic buffer is listed in Table 1, page 75.

TABLE 1

Absorbance-Concentration Data For Iron (III)/Hydroxamate Complexes in a Citric Acid-Sodium Phosphate Dibasic Buffer

Concentration of the Iron (III)/ Hydroxamate Complex (mole/liter)	Absorbance
1.8×10^{-4}	0.15
3.6×10^{-4}	0.30
5.4×10^{-4}	0.46
7.3×10^{-4}	0.62
9.1×10^{-4}	0.77

APPENDIX B

EXPERIMENTAL RESULTS

B.1 Development of a Hydrogen Peroxide Calibration Curve

The absorbance/concentration data for standardized H_2O_2 solutions is listed in Table 2, page 77. The data was put into a least squares fit program to determine the molar extinction coefficient of H_2O_2. The molar extinction coefficient was found to be 40.05 l/cm mole (at 240 nm).*

A graph of absorbance versus concentration for the H_2O_2 solutions is shown in Figure 26, page 78. The line passing through the data points is the line calculated by a least squares fit program. As shown, there is almost no deviation of the data points from the least squares fit line; the coefficient of fit for the straight line was 0.99. This indicates that throughout the development of the calibration curve, good precision was maintained.

---

*See page 28 for a comparison between the above molar extinction coefficient and those listed in the literature.

TABLE 2

Development of a Hydrogen Peroxide Calibration Curve; Absorbance-Concentration Data*

Concentration (mole/liter)	Absorbance
1.00×10^{-2}	0.386
1.25×10^{-2}	0.487
2.00×10^{-2}	0.787
2.50×10^{-2}	0.987

*The H_2O_2 solutions were kept at pH = 7.0 and 298°K. The absorbances were read at 240 nm.

FIGURE 26. Hydrogen Peroxide Calibration Curve (240 nm)

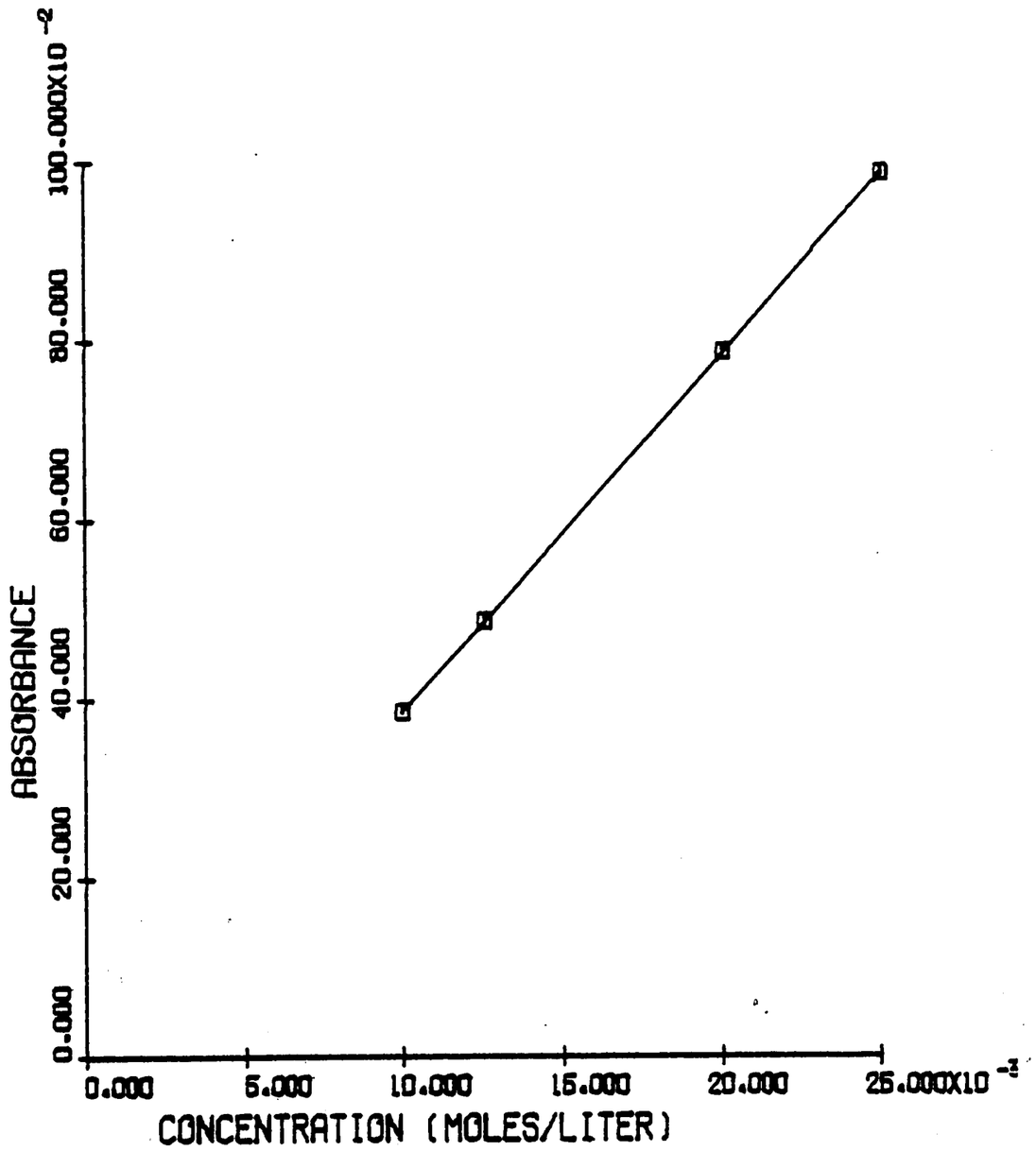


TABLE 3

Experimental Results of The Catalase Activity Assay

Trial #	Time Required For H_2O_2 Concentration to Go From 10.3 µmole/ml to 9.2 µmole/ml (minutes)	Calculated Catalase Activity (units/ml)
1	6.20	.177
2	6.02	.183
3	5.78	.190
4	6.20	.177
5	6.07	.181
6	5.75	.191

Note: 1) The catalase activity is calculated by dividing the change in hydrogen peroxide concentration (in µmole/ml) by the time (in minutes) required to make this change. The catalase activity was determined at pH = 7.0 and 25°C.

2) From the above data, the average catalase activity was calculated to be .183 units/ml.

3) The catalase activities listed above are for a 5% solution of the glucose oxidase preparation.

TABLE 4

Precision Analysis of The Catalase Activity Assay

Average Catalase Activity = .183 units/ml*

Trial #	Calculated Catalase Activity (units/ml)	Percent Deviation From The Mean (%)
1	.177	3.3
2	.183	0.0
3	.190	3.8
4	.177	3.3
5	.181	1.1
6	.191	4.4

*The catalase activity (.183 units/ml) is for a 5% solution of the glucose oxidase preparation. Thus, for the undiluted glucose oxidase preparation, the activity would be 3.7 units/ml.

TABLE 5

Determination of The Rate Constant For The

Decomposition of H_2O_2 Via Catalase-Experimental Data

Absorbance Data

Trial #	Initial Absorbance (A_1)	Final Absorbance (A_2)	*Calculated Rate Constant (min^{-1})
1	0.780 0.783 (0.782)** 0.782	0.736 0.738 (0.737)** 0.738	.0395
2	0.924 0.919 (0.920) 0.917	0.868 0.866 (0.867) 0.868	.0396
3	0.803 0.799 (0.800) 0.798	0.755 0.754 (0.755) 0.757	.0386
4	0.709 0.708 (0.709) 0.710	0.667 0.666 (0.666) 0.664	.0417

The above rate constants are for a 10% glucose oxidase solution.

---

*The rate constant is calculated according to equation 10, page 10.

**The numbers in parentheses indicate an average initial (or final) absorbance value.

TABLE 5
(continued)

Determination of The Rate Constant For The

Decomposition of H_2O_2 Via Catalase-Experimental Data

The average catalase rate constant for a 10% glucose oxidase solution is $k = .0399\ min^{-1}$. The percent deviations from the mean for the various trials are listed below.

Trial #	Percent Deviation From The Mean (%)
1	1.0
2	0.8
3	3.3
4	4.5

Note: 1) The above rate constants were determined at pH = 5.5 (0.5 M sodium acetate buffer) and 30°C. The absorbances were measured at 240 nm.

2) Since $k = .0399\ min^{-1}$ for a 10% glucose oxidase solution, then for the undiluted glucose oxidase preparation, $k = 0.399\ min^{-1}$.

TABLE 6

Preparation of a Gluconolactone Calibration Curve:

Absorbance-Concentration Data For

Iron (III)/Hydroxamate Solutions

Concentration of The Iron (III)/ Hydroxamate Solution (moles/liter)*	Absorbance
2.0×10^{-4}	0.18
4.0×10^{-4}	0.33
6.0×10^{-4}	0.49
8.0×10^{-4}	0.72
10.0×10^{-4}	0.92

*The absorbances of the iron (III)/hydroxamate solutions were determined at 540 nm.

FIGURE 27. Gluconolactone Calibration Curve (540 nm)

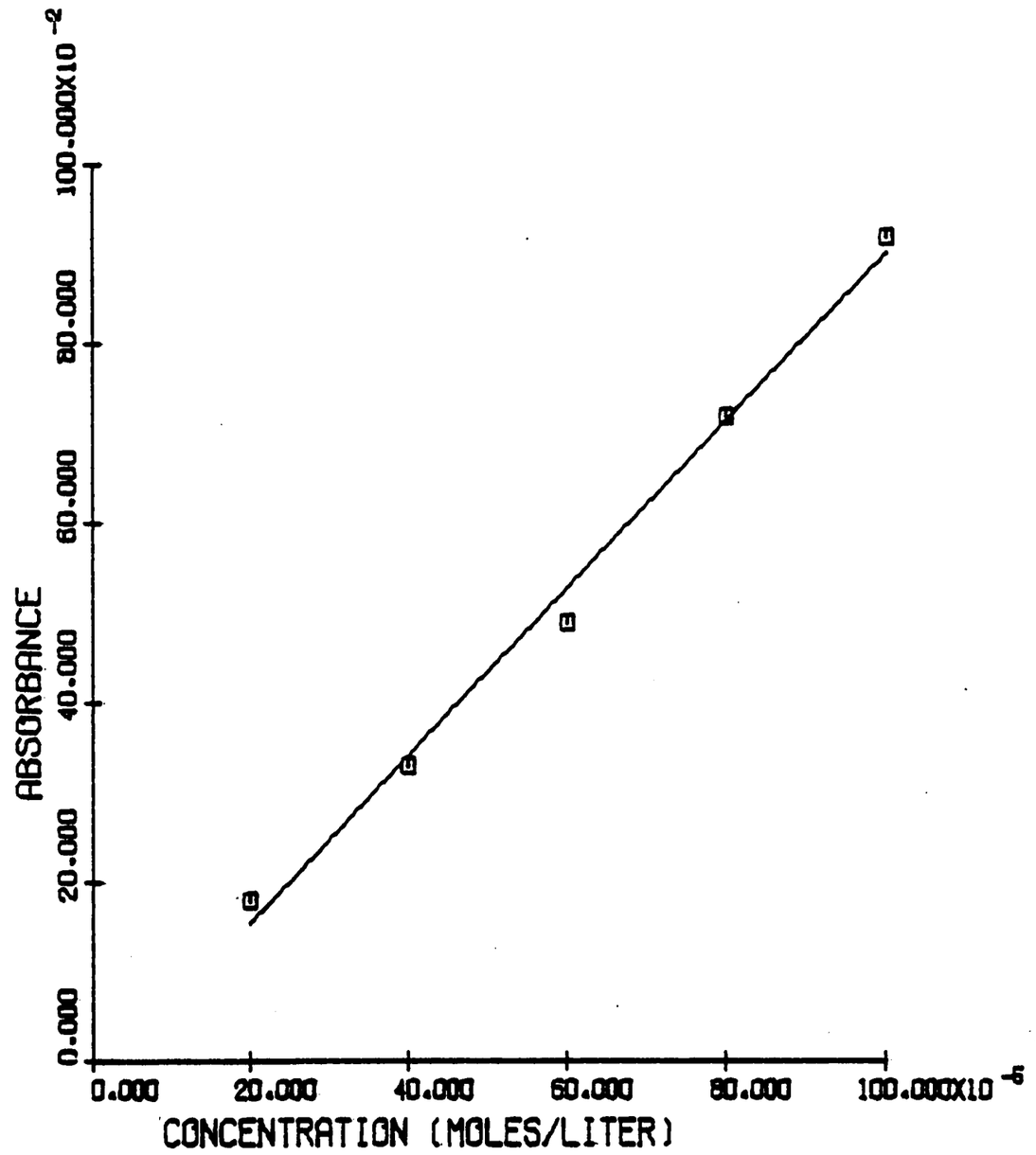


TABLE 7

Data For Determining The Rate Constants of
The Spontaneous and Enzymatic Hydrolysis of
Gluconolactone* in 0.5 M Sodium Acetate Buffer

0% Enzyme Solution - Spontaneous Hydrolysis

Trial I		Trial II	
Time (min)	Absorbance	Time (min)	Absorbance
5	0.683	5	0.710
10	0.635	10	0.618
15	0.566	16	0.530
20	0.518	20	0.518
25	0.472	25	0.462
30	0.425	30	0.430
35	0.402	35	0.393
40	0.378	40	0.374

4% Enzyme Solution

Trial I		Trial II	
Time (min)	Absorbance	Time (min)	Absorbance
1	0.758	1	0.784
2	0.631	2	0.705
4	0.555	4	0.668
8	0.463	8	0.520
10	0.380	10	0.426

*The reaction conditions were pH = 5.5 (0.5 M sodium acetate buffer) and temperature = 30°C.

TABLE 7
(continued)

Data For Determining The Rate Constants of
The Spontaneous and Enzymatic Hydrolysis of
Gluconolactone in 0.5 M Sodium Acetate Buffer

8% Enzyme Solution

Trial I		Trial II	
Time (min)	Absorbance	Time (min)	Absorbance
1	0.742	1	0.768
2	0.670	2	0.657
4	0.508	4	0.516
8	0.324	8	0.338
10	0.282	10	0.272

Notes: 1) The above data is plotted in the form of ln (absorbance) versus time (refer to Figures 28-30, pages 87-89). The rate constants for each of the solutions are determined from the slopes of the lines of the resulting graphs.

2) The rate constants determined from Figures 29 and 30 (the 4% and 8% enzyme solutions) are overall rate constants (refer to page 8). The enzymatic rate constants are determined by subtracting the spontaneous rate constant* from each of the overall rate constants.

*The spontaneous rate constant is determined from Figure 28.

FIGURE 28. The Hydrolysis of Gluconolactone in a Sodium Acetate Buffer (pH = 5.5) at 30°C; 0% Enzyme Solution

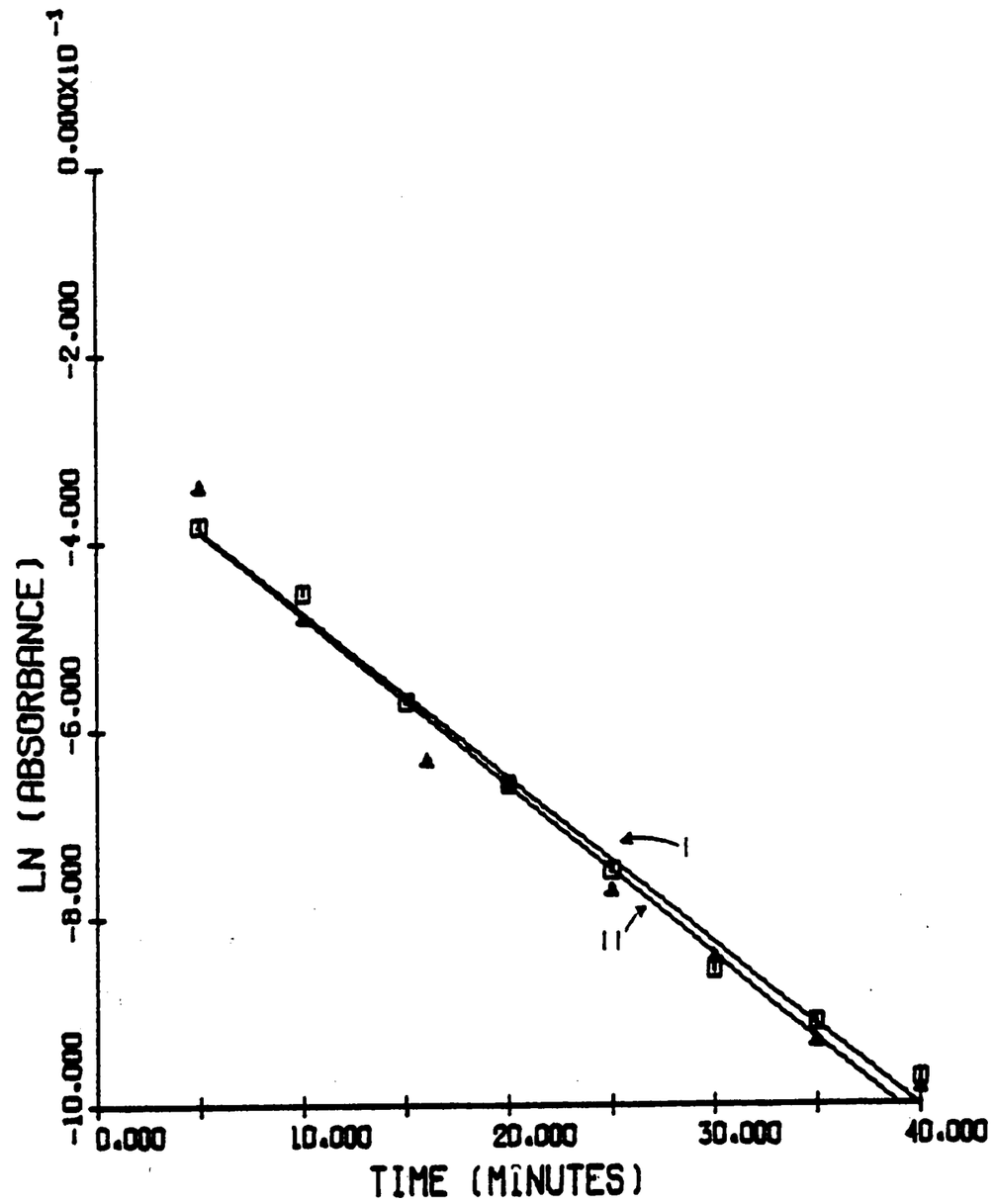


FIGURE 29. The Hydrolysis of Gluconolactone in a Sodium Acetate Buffer (pH = 5.5) at 30°C; 4% Enzyme Solution

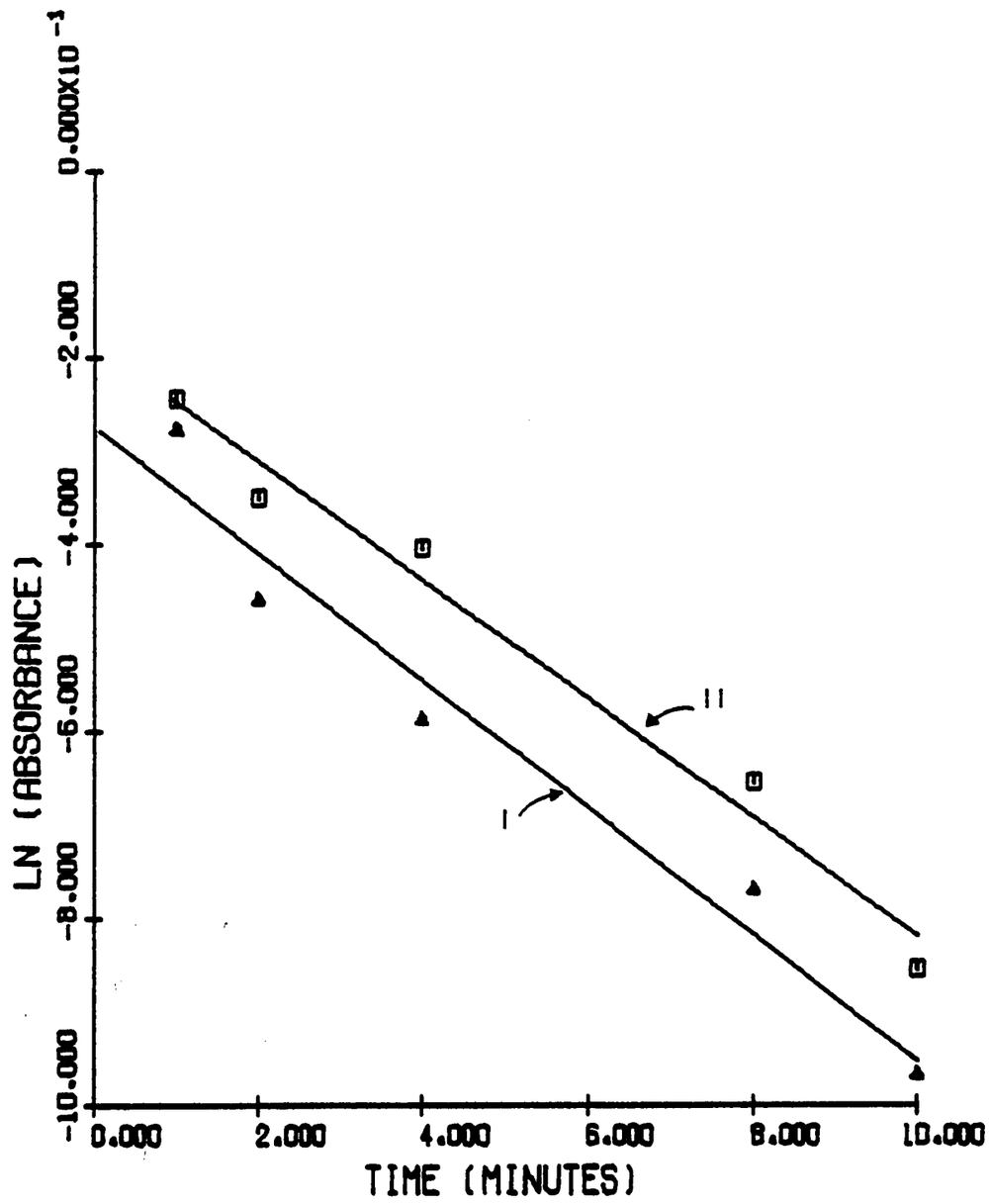


FIGURE 30. The Hydrolysis of Gluconolactone in a Sodium Acetate Buffer (pH = 5.5) at 30°C; 8% Enzyme Solution

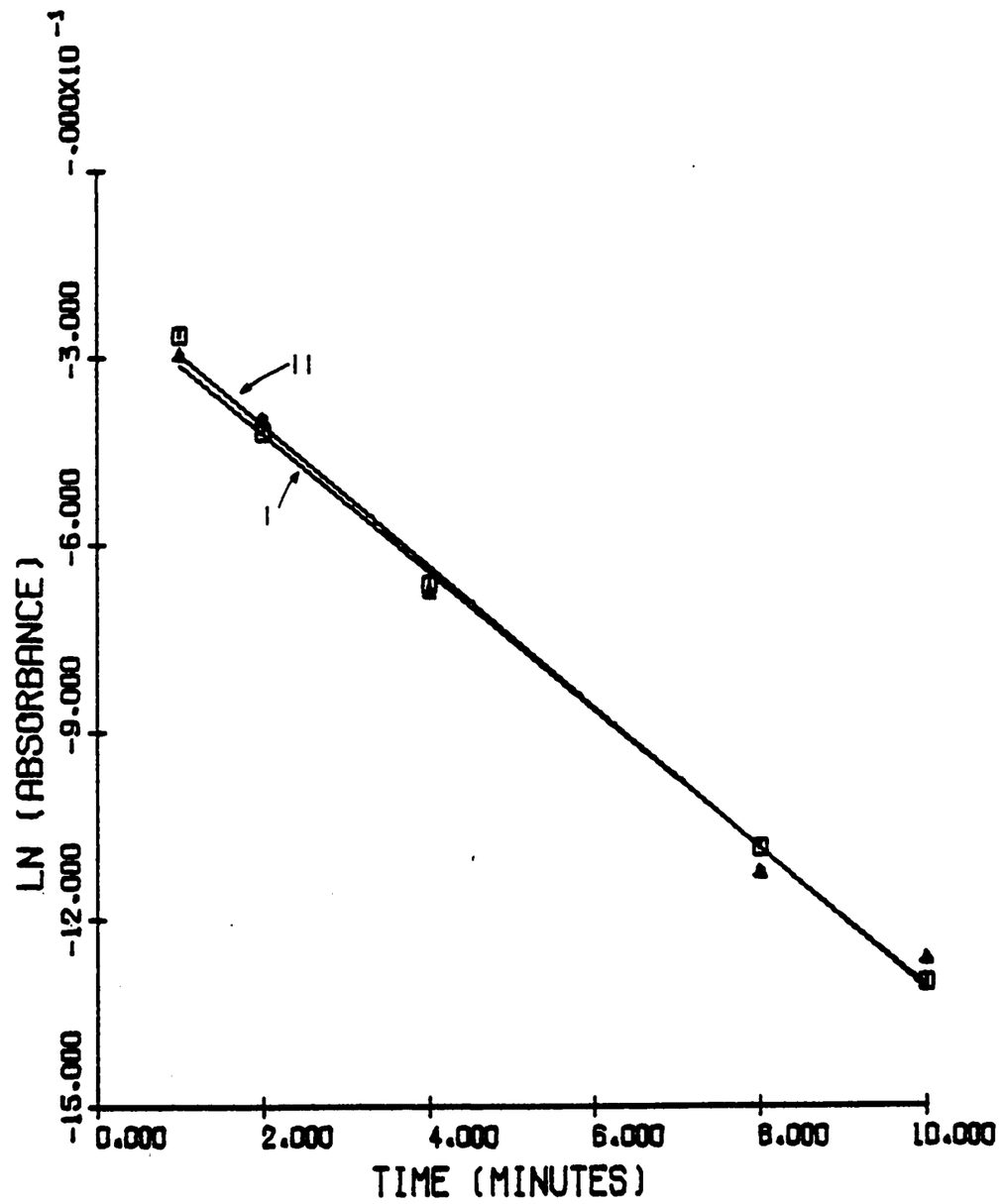


TABLE 8

Rate Constants For The Spontaneous and Enzymatic Hydrolysis of Gluconolactone in a 0.5 M Sodium Acetate Buffer (pH = 5.5) at 30°C

Concentration of Enzyme Solution	Rate Constant (min^{-1}) Trial I	Trial II	Average Rate Constant (min^{-1})	% Deviation From Mean
0%	.0176	.0180	.0178	1.1
4%	.0500	.0456	.0478	4.6
8%	.0928	.0953	.0941	1.3

Notes: 1) The activities of glucose oxidase, in terms of units glucose oxidase per liter, for the various solutions are:

Enzyme Solution	Activity of Glucose Oxidase (units G. Oxidase/liter)
0%	0
4%	1.1×10^5
8%	2.2×10^5

2) The rate constant/concentration data was put into a least squares fit program and the enzymatic rate constant for the 100% (undiluted) glucose oxidase preparation was found to be $k_e = 1.158\ min^{-1}$.

TABLE 9

Results of The Analysis of H_2O_2, Gluconolactone, and Gluconic Acid Solutions Using The YSI Glucose Analyzer

A) Gluconic Acid Analysis

Concentration of Gluconic Acid Solution (%)	Glucose Analyzer Reading (mg/dl)
10	0
25	0

B) Gluconolactone Analysis

Concentration of Gluconolactone Solution (moles/liter)	Glucose Analyzer Reading (mg/dl)
1.0	19
0.1	2
0.01	0

C) Hydrogen Peroxide Analysis

Hydrogen Peroxide Concentration (moles/liter)	Expected Glucose Analyzer Readout (mg/dl)	Actual Glucose Analyzer Readout (mg/dl)		
		Trial I	Trial II	Trial III
.011	200	114	80	70
5.5×10^{-3}	100	48	33	30

Notes: 1) The hydrogen peroxide solutions were standardized using the procedure listed on page 71.

TABLE 9
(continued)

Results of The Analysis of H_2O_2, Gluconolactone,

and Gluconic Acid Solutions Using The YSI Glucose Analyzer

Notes: 2) The reaction occurring in the glucose analyzer is the following:

$$\text{glucose} + H_2O + O_2 \xrightarrow[\text{oxidase}]{\text{glucose}} H_2O_2 + \text{gluconic acid}$$

The stoichiometry of the reaction indicates that a .011 M solution of glucose (200 mg/dl of glucose) is converted into .011 M H_2O_2. Since the glucose analyzer measures glucose concentration indirectly by measuring H_2O_2 concentration, it is apparent that a .011 M H_2O_2 solution should give the same result (200 mg/dl reading by the analyzer) as a .011 M solution of glucose. Likewise, analysis of a 5.5×10^{-3} M H_2O_2 solution should result in an analyzer readout of 100 mg/dl. Hence, the heading "Expected Glucose Analyzer Readout."

3) For the H_2O_2 tests, the time difference between each of the trials (I, II, III) was five minutes; a time interval long enough in which to recalibrate the glucose analyzer.

B.2 Precision and Accuracy Data For a Five Milliliter Mohr Pipette

Tests were conducted on a 5.0 ml Mohr pipette to determine the accuracy and precision with which it delivers a 1.0 ml sample of water. The tests involved weighing various water samples which, according to the graduations on the Mohr pipette, had a volume of 1.0 ml. By measuring the temperature of the samples, the density was determined, and from the density and mass data, the actual volume of water was calculated. The data obtained from these tests is listed in Table 10.

TABLE 10

Temperature of water = 21°C Density of water = .998 gm/ml (@21°C)

Volume of Water as Indicated by Pipette Graduations, (ml)	Mass of Water Sample, (grams)	Actual Volume of Water, (ml)	Precision (% Deviation From Average Volume of, .9975 ml)	Accuracy (Relative Error, %)
1.0	.9905	.9925	0.5%	-.75%
1.0	.9968	.9988	0.13%	-.12%
1.0	.9991	1.0011	0.36%	+.11%

B.3 Precision and Accuracy Data For The Clay Adams Adjustable Syringe

An experiment was conducted to determine the accuracy and precision with which an adjustable syringe (Clay Adams) delivers a 1.0 ml sample of water. The data obtained from this experiment is listed in Table 11.

TABLE 11

Volume of H_2O as Indicated by Syringe Graduations, (ml)	Mass of Water Sample, (grams)	Actual Volume of Water, (ml)	Precision (% Deviation From Average Volume of 1.009 ml)	Accuracy (Relative Error)
1.0	1.0024	1.004	0.49	+0.4
1.0	1.0111	1.013	0.40	+1.3
1.0	1.0067	1.009	0.00	+0.9
1.0	1.0080	1.010	0.10	+1.0
1.0	1.0067	1.009	0.00	+0.9
1.0	1.0084	1.010	0.10	+1.0

APPENDIX C

ERROR ANALYSIS

C.1 The Effect That Mohr Pipettes Have on The Precision of The Catalase Activity Assay

Mohr pipettes can deliver liquids with an average precision of 0.33% deviation from the mean (see Note 1). Thus, when pipetting a 0.5 ml sample (as in the catalase activity assay, see page 17) it is possible to deliver volumes as high as 0.502 ml or as low as 0.498 ml.

The use of Mohr pipettes can result in different catalase activity values. For example, if 0.498 ml of enzyme solution (see Note 2) is combined with 0.502 ml of phosphate buffer, the catalase activity within the cuvette would be 0.182 units/ml. On the other hand, if 0.502 ml of enzyme solution is combined with 0.498 ml of buffer, the resultant activity would be 0.184 units/ml.

The average catalase activity in the cuvette is 0.183 units/ml; the precision, in terms of percent deviation from the mean, is 0.5%. Therefore, the use of Mohr pipettes can result in differences in the catalase activity within the cuvette (which, in turn, would lead to the calculation of different catalase activity values for the glucose oxidase preparation). However, the extent to which these differences contribute to the overall precision of the assay is difficult to assess due to other problems encountered in the assay (see page 29).

Notes:

1) Precision and accuracy data for a 1.0 ml Mohr pipet is listed in Table 10, page 93. Mohr pipettes were used for the catalase activity because they allowed for a faster and easier transfer of solutions than did volumetric pipettes.
2) In the above analysis, the catalase activity of the enzyme solution (determined experimentally) is .366 units/ml.

C.2 Mohr Pipettes and How They Affect The Precision of The Lactone Hydrolysis Rate Constant Determination*

In the gluconolactone assay (see page 23), 7.0 ml of solutions (4.0 ml hydroxylamine reagent, 2.0 ml 4 M HCl, 1.0 ml $FeCl_3$ solution) are transferred using Mohr pipettes. The sample is transferred using a 1.0 ml syringe.**

If the Mohr pipettes are used in such a way that the lowest precision (0.5% deviation from the mean) is obtained (see Note 1), then for a solution size of 7.0 ml, the maximum volume delivered would be 7.035 ml; the minimum volume delivered would be 6.965 ml (see Note 2).

At a reaction time of five minutes, 1.0 ml of 7.3×10^{-4} M lactone sample (see Note 3) is added to both the 7.035 ml solution and the 6.965 ml solution; the resultant lactone concentrations are 9.09×10^{-5} M and 9.17×10^{-5} M, respectively. Likewise, at a

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*Hereafter referred to as the gluconolactone assay.

**The syringe used was a 1.0 ml adjustable syringe (Clay Adams). See page 93 for information regarding the accuracy and precision of this syringe.

reaction time of forty minutes, 1.0 ml of a 4.04×10^{-4} M lactone sample is transferred to both the 7.035 ml solution and the 6.965 ml solution. The lactone concentrations are 5.03×10^{-5} M and 5.07×10^{-5} M, respectively. The concentrations of the solutions, as well as their respective sample times, are given in Table 12.

TABLE 12

Data Point	Time (min)	Lactone Concentration (M)	ln (Lactone Concentration)
A	5	9.17×10^{-5}	-9.30
B	5	9.09×10^{-5}	-9.31
C	40	5.07×10^{-5}	-9.89
D	40	5.03×10^{-5}	-9.90

The largest difference in rate constants would occur when data points A and D represent the data from one assay and data points B and C represent the data from a second assay (see Note 2). The rate constants for each of the "assays" would then be:

assay 1: (data points A+D) $k_1 = \frac{-9.90 - (-9.30)}{40 - 5} = -.0171 \text{ min}^{-1}$

assay 2: (data points B+C) $k_2 = \frac{-9.89 - (-9.31)}{40 - 5} = -.0166 \text{ min}^{-1}$

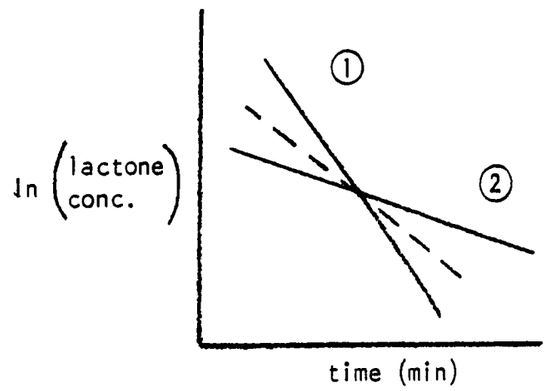
The average rate constant is $k = .0169 \text{ min}^{-1}$. The percent deviation from the mean is 1.48%.

The least precision obtained when using the Mohr pipettes is one whereby the percent deviation from the mean is 1.48%.

Notes:

1) Data concerning the accuracy and precision of Mohr pipettes is listed in Table 10, page 93.

2) In the above analysis, two hypothetical assays are performed from which a maximum and a minimum rate constant are found. The analysis is done in such a way as to present an "extreme" situation; that is, one in which there is a maximum difference between the rate constants. This situation is represented in the figure below where the (natural) log of the lactone concentration is plotted as a function of time.



The lactone hydrolysis rate constants, being first order, are determined from the slopes of the various lines. Lines one and two result from plotting the data obtained from their respective assays. Obviously, line one provides a larger rate constant than does line two. The dotted line represents the rate constant obtained by averaging the results of lines one and two.

3) The lactone concentrations used in this analysis are data obtained during an actual rate constant determination.* The lactone concentrations were determined from their respective absorbance data by using the molar extinction coefficient of the iron (III)/hydroxamate complex.

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*Refer to Table 7, page 85.

APPENDIX D

THE COMPUTER-GENERATED CONCENTRATION DATA FOR THE SUBSTITUENTS IN A CLOSED GLUCOSE OXIDASE REACTION SYSTEM

D.1 Computer-Generated Concentration Data For The Substituents in a Closed Glucose Oxidase Reaction System

Notes concerning Tables 13 through 48.

1) The computer data is for a "reaction system" having a volume of 300.0 ml and maintained at pH = 5.5 and T = 30°C.

2) The concentrations (in moles/liter) of the substituents listed in Tables 13 through 48 are represented by the following symbols:

S = Glucose

HP = Hydrogen Peroxide

GL = Gluconolactone

GA = Gluconic Acid

OX = Oxygen

3) Other symbols used in Tables 13 to 48 are:

T = reaction time (minutes)

X = quantity of glucose oxidase preparation (milliliters)

ACT = glucose oxidase activity in the system expressed in moles/l minute.

TABLE 13

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E-01 ACT= 9.170E-04 Catalase Activity = 1.23×10^{-3} units/ml

T	S	HP	GL	GA	OX
0.	1.000E-02	0.	0.	0.	2.500E-04
3.750E-01	9.966E-03	3.384E-05	3.373E-05	1.158E-07	2.162E-04
7.500E-01	9.934E-03	6.646E-05	6.600E-05	4.567E-07	1.835E-04
1.125E+00	9.902E-03	9.759E-05	9.659E-05	1.012E-06	1.524E-04
1.500E+00	9.873E-03	1.269E-04	1.252E-04	1.770E-06	1.231E-04
1.875E+00	9.846E-03	1.540E-04	1.513E-04	2.714E-06	9.598E-05
2.250E+00	9.822E-03	1.783E-04	1.745E-04	3.827E-06	7.164E-05
2.625E+00	9.801E-03	1.993E-04	1.943E-04	5.087E-06	5.067E-05
3.000E+00	9.784E-03	2.163E-04	2.099E-04	6.467E-06	3.363E-05
3.375E+00	9.771E-03	2.291E-04	2.213E-04	7.940E-06	2.084E-05
3.750E+00	9.762E-03	2.379E-04	2.285E-04	9.476E-06	1.209E-05
4.125E+00	9.757E-03	2.433E-04	2.323E-04	1.105E-05	6.648E-06
4.500E+00	9.753E-03	2.464E-04	2.339E-04	1.264E-05	3.522E-06
4.875E+00	9.752E-03	2.481E-04	2.340E-04	1.424E-05	1.825E-06
5.250E+00	9.751E-03	2.490E-04	2.333E-04	1.583E-05	9.355E-07
5.625E+00	9.750E-03	2.495E-04	2.322E-04	1.742E-05	4.784E-07
6.000E+00	9.750E-03	2.497E-04	2.308E-04	1.900E-05	2.459E-07
6.375E+00	9.750E-03	2.498E-04	2.294E-04	2.056E-05	1.284E-07
6.750E+00	9.750E-03	2.498E-04	2.279E-04	2.212E-05	6.912E-08
7.125E+00	9.750E-03	2.499E-04	2.264E-04	2.367E-05	3.927E-08
7.500E+00	9.750E-03	2.499E-04	2.249E-04	2.521E-05	2.426E-08
7.875E+00	9.750E-03	2.499E-04	2.233E-04	2.674E-05	1.670E-08
8.250E+00	9.750E-03	2.499E-04	2.218E-04	2.826E-05	1.290E-08
8.625E+00	9.750E-03	2.499E-04	2.203E-04	2.977E-05	1.099E-08
9.000E+00	9.750E-03	2.499E-04	2.188E-04	3.126E-05	1.003E-08
9.375E+00	9.750E-03	2.499E-04	2.174E-04	3.275E-05	9.550E-09
9.750E+00	9.750E-03	2.499E-04	2.159E-04	3.423E-05	9.307E-09
1.012E+01	9.750E-03	2.498E-04	2.144E-04	3.570E-05	9.185E-09
1.050E+01	9.750E-03	2.498E-04	2.130E-04	3.715E-05	9.123E-09
1.087E+01	9.750E-03	2.498E-04	2.115E-04	3.860E-05	9.092E-09
1.125E+01	9.750E-03	2.498E-04	2.101E-04	4.004E-05	9.076E-09
1.162E+01	9.750E-03	2.498E-04	2.087E-04	4.147E-05	9.068E-09
1.200E+01	9.750E-03	2.498E-04	2.073E-04	4.288E-05	9.064E-09
1.237E+01	9.750E-03	2.498E-04	2.059E-04	4.429E-05	9.062E-09
1.275E+01	9.750E-03	2.498E-04	2.045E-04	4.569E-05	9.061E-09
1.313E+01	9.750E-03	2.498E-04	2.031E-04	4.708E-05	9.060E-09
1.350E+01	9.750E-03	2.498E-04	2.017E-04	4.846E-05	9.059E-09
1.387E+01	9.750E-03	2.498E-04	2.004E-04	4.983E-05	9.059E-09
1.425E+01	9.750E-03	2.498E-04	1.990E-04	5.120E-05	9.059E-09
1.462E+01	9.750E-03	2.498E-04	1.977E-04	5.255E-05	9.058E-09
1.500E+01	9.750E-03	2.498E-04	1.963E-04	5.389E-05	9.058E-09

TABLE 14

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X = 1.000E-01 ACT = 9.170E-04 Catalase Activity = 1.23×10^{-3} units/ml

T	S	HP	GL	GA	OX
0.	1.000E-02	0.	0.	0.	1.250E-03
3.750E-01	9.959E-03	4.083E-05	4.070E-05	1.390E-07	1.209E-03
7.500E-01	9.919E-03	8.146E-05	8.091E-05	5.540E-07	1.169E-03
1.125E+00	9.878E-03	1.219E-04	1.206E-04	1.242E-06	1.128E-03
1.500E+00	9.838E-03	1.621E-04	1.599E-04	2.198E-06	1.088E-03
1.875E+00	9.798E-03	2.021E-04	1.987E-04	3.421E-06	1.048E-03
2.250E+00	9.758E-03	2.418E-04	2.370E-04	4.907E-06	1.008E-03
2.625E+00	9.719E-03	2.814E-04	2.748E-04	6.652E-06	9.686E-04
3.000E+00	9.679E-03	3.207E-04	3.121E-04	8.654E-06	9.293E-04
3.375E+00	9.640E-03	3.598E-04	3.489E-04	1.091E-05	8.902E-04
3.750E+00	9.601E-03	3.986E-04	3.853E-04	1.341E-05	8.514E-04
4.125E+00	9.563E-03	4.372E-04	4.211E-04	1.616E-05	8.128E-04
4.500E+00	9.524E-03	4.755E-04	4.565E-04	1.915E-05	7.744E-04
4.875E+00	9.486E-03	5.136E-04	4.914E-04	2.239E-05	7.363E-04
5.250E+00	9.448E-03	5.514E-04	5.257E-04	2.586E-05	6.985E-04
5.625E+00	9.411E-03	5.889E-04	5.596E-04	2.956E-05	6.610E-04
6.000E+00	9.374E-03	6.261E-04	5.929E-04	3.349E-05	6.238E-04
6.375E+00	9.337E-03	6.630E-04	6.257E-04	3.764E-05	5.868E-04
6.750E+00	9.300E-03	6.996E-04	6.579E-04	4.202E-05	5.502E-04
7.125E+00	9.264E-03	7.358E-04	6.896E-04	4.661E-05	5.140E-04
7.500E+00	9.228E-03	7.717E-04	7.207E-04	5.142E-05	4.781E-04
7.875E+00	9.192E-03	8.072E-04	7.512E-04	5.644E-05	4.426E-04
8.250E+00	9.157E-03	8.422E-04	7.810E-04	6.167E-05	4.076E-04
8.625E+00	9.123E-03	8.767E-04	8.102E-04	6.709E-05	3.730E-04
9.000E+00	9.089E-03	9.108E-04	8.386E-04	7.272E-05	3.390E-04
9.375E+00	9.055E-03	9.442E-04	8.663E-04	7.853E-05	3.055E-04
9.750E+00	9.022E-03	9.770E-04	8.931E-04	8.453E-05	2.727E-04
1.012E+01	8.990E-03	1.009E-03	9.190E-04	9.071E-05	2.406E-04
1.050E+01	8.959E-03	1.040E-03	9.438E-04	9.706E-05	2.095E-04
1.087E+01	8.929E-03	1.070E-03	9.675E-04	1.036E-04	1.794E-04
1.125E+01	8.900E-03	1.099E-03	9.897E-04	1.103E-04	1.505E-04
1.162E+01	8.873E-03	1.126E-03	1.010E-03	1.171E-04	1.231E-04
1.200E+01	8.847E-03	1.152E-03	1.029E-03	1.240E-04	9.755E-05
1.237E+01	8.824E-03	1.175E-03	1.045E-03	1.311E-04	7.431E-05
1.275E+01	8.803E-03	1.196E-03	1.058E-03	1.383E-04	5.388E-05
1.313E+01	8.786E-03	1.213E-03	1.068E-03	1.455E-04	3.682E-05
1.350E+01	8.773E-03	1.226E-03	1.074E-03	1.528E-04	2.353E-05
1.387E+01	8.763E-03	1.235E-03	1.076E-03	1.602E-04	1.406E-05
1.425E+01	8.757E-03	1.241E-03	1.075E-03	1.675E-04	7.918E-06
1.462E+01	8.754E-03	1.245E-03	1.072E-03	1.748E-04	4.275E-06
1.500E+01	8.752E-03	1.247E-03	1.066E-03	1.821E-04	2.253E-06

TABLE 15

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X = 1.000E-01 ACT = 9.170E-04 Catalase Activity = 1.23×10^{-3} units/ml

T	S	HP	GL	GA	OX
0.	1.000E-02	0.	0.	0.	2.500E-03
3.750E-01	9.958E-03	4.185E-05	4.171E-05	1.425E-07	2.458E-03
7.500E-01	9.916E-03	8.353E-05	8.297E-05	5.679E-07	2.416E-03
1.125E+00	9.875E-03	1.250E-04	1.238E-04	1.273E-06	2.375E-03
1.500E+00	9.834E-03	1.664E-04	1.642E-04	2.255E-06	2.334E-03
1.875E+00	9.792E-03	2.076E-04	2.041E-04	3.511E-06	2.292E-03
2.250E+00	9.751E-03	2.486E-04	2.436E-04	5.038E-06	2.251E-03
2.625E+00	9.711E-03	2.894E-04	2.826E-04	6.832E-06	2.211E-03
3.000E+00	9.670E-03	3.301E-04	3.213E-04	8.892E-06	2.170E-03
3.375E+00	9.629E-03	3.706E-04	3.595E-04	1.121E-05	2.129E-03
3.750E+00	9.589E-03	4.109E-04	3.972E-04	1.379E-05	2.089E-03
4.125E+00	9.549E-03	4.511E-04	4.346E-04	1.663E-05	2.049E-03
4.500E+00	9.509E-03	4.911E-04	4.715E-04	1.972E-05	2.009E-03
4.875E+00	9.469E-03	5.309E-04	5.080E-04	2.306E-05	1.969E-03
5.250E+00	9.429E-03	5.706E-04	5.441E-04	2.665E-05	1.929E-03
5.625E+00	9.390E-03	6.101E-04	5.798E-04	3.048E-05	1.890E-03
6.000E+00	9.350E-03	6.494E-04	6.151E-04	3.456E-05	1.850E-03
6.375E+00	9.311E-03	6.885E-04	6.500E-04	3.887E-05	1.811E-03
6.750E+00	9.272E-03	7.275E-04	6.844E-04	4.342E-05	1.772E-03
7.125E+00	9.233E-03	7.663E-04	7.185E-04	4.820E-05	1.733E-03
7.500E+00	9.195E-03	8.050E-04	7.522E-04	5.322E-05	1.695E-03
7.875E+00	9.156E-03	8.435E-04	7.854E-04	5.846E-05	1.656E-03
8.250E+00	9.118E-03	8.818E-04	8.183E-04	6.393E-05	1.618E-03
8.625E+00	9.080E-03	9.199E-04	8.508E-04	6.962E-05	1.580E-03
9.000E+00	9.042E-03	9.579E-04	8.829E-04	7.553E-05	1.542E-03
9.375E+00	9.004E-03	9.957E-04	9.146E-04	8.166E-05	1.504E-03
9.750E+00	8.966E-03	1.033E-03	9.460E-04	8.801E-05	1.466E-03
1.012E+01	8.929E-03	1.071E-03	9.769E-04	9.457E-05	1.429E-03
1.050E+01	8.891E-03	1.108E-03	1.007E-03	1.013E-04	1.392E-03
1.087E+01	8.854E-03	1.145E-03	1.038E-03	1.083E-04	1.354E-03
1.125E+01	8.817E-03	1.182E-03	1.067E-03	1.155E-04	1.317E-03
1.162E+01	8.780E-03	1.219E-03	1.097E-03	1.229E-04	1.281E-03
1.200E+01	8.744E-03	1.255E-03	1.126E-03	1.304E-04	1.244E-03
1.237E+01	8.707E-03	1.292E-03	1.155E-03	1.382E-04	1.208E-03
1.275E+01	8.671E-03	1.328E-03	1.183E-03	1.462E-04	1.171E-03
1.313E+01	8.635E-03	1.364E-03	1.211E-03	1.544E-04	1.135E-03
1.350E+01	8.599E-03	1.400E-03	1.238E-03	1.627E-04	1.099E-03
1.387E+01	8.563E-03	1.436E-03	1.266E-03	1.712E-04	1.064E-03
1.425E+01	8.527E-03	1.471E-03	1.293E-03	1.800E-04	1.028E-03
1.462E+01	8.492E-03	1.506E-03	1.319E-03	1.889E-04	9.929E-04
1.500E+01	8.457E-03	1.541E-03	1.345E-03	1.980E-04	9.577E-04

TABLE 16

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E-01 ACT= 9.170E-04 Catalase Activity = 1.23×10^{-3} units/ml

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	2.500E-04
3.750E-01	9.992E-02	8.321E-05	8.292E-05	2.943E-07	1.668E-04
7.500E-01	9.985E-02	1.461E-04	1.451E-04	1.084E-06	1.039E-04
1.125E+00	9.981E-02	1.895E-04	1.873E-04	2.227E-06	6.051E-05
1.500E+00	9.978E-02	2.166E-04	2.130E-04	3.600E-06	3.337E-05
1.875E+00	9.977E-02	2.323E-04	2.272E-04	5.107E-06	1.770E-05
2.250E+00	9.976E-02	2.408E-04	2.342E-04	6.683E-06	9.163E-06
2.625E+00	9.975E-02	2.453E-04	2.371E-04	8.292E-06	4.682E-06
3.000E+00	9.975E-02	2.476E-04	2.377E-04	9.912E-06	2.377E-06
3.375E+00	9.975E-02	2.488E-04	2.373E-04	1.153E-05	1.204E-06
3.750E+00	9.975E-02	2.493E-04	2.363E-04	1.315E-05	6.110E-07
4.125E+00	9.975E-02	2.496E-04	2.350E-04	1.475E-05	3.120E-07
4.500E+00	9.975E-02	2.498E-04	2.335E-04	1.635E-05	1.614E-07
4.875E+00	9.975E-02	2.498E-04	2.320E-04	1.794E-05	8.567E-08
5.250E+00	9.975E-02	2.499E-04	2.305E-04	1.952E-05	4.758E-08
5.625E+00	9.975E-02	2.499E-04	2.290E-04	2.108E-05	2.843E-08
6.000E+00	9.975E-02	2.499E-04	2.274E-04	2.264E-05	1.880E-08
6.375E+00	9.975E-02	2.499E-04	2.259E-04	2.419E-05	1.396E-08
6.750E+00	9.975E-02	2.499E-04	2.244E-04	2.572E-05	1.152E-08
7.125E+00	9.975E-02	2.499E-04	2.228E-04	2.725E-05	1.030E-08
7.500E+00	9.975E-02	2.499E-04	2.213E-04	2.876E-05	9.683E-09
7.875E+00	9.975E-02	2.499E-04	2.198E-04	3.026E-05	9.373E-09
8.250E+00	9.975E-02	2.499E-04	2.184E-04	3.176E-05	9.218E-09
8.625E+00	9.975E-02	2.499E-04	2.169E-04	3.324E-05	9.139E-09
9.000E+00	9.975E-02	2.499E-04	2.154E-04	3.472E-05	9.100E-09
9.375E+00	9.975E-02	2.498E-04	2.140E-04	3.618E-05	9.080E-09
9.750E+00	9.975E-02	2.498E-04	2.125E-04	3.764E-05	9.070E-09
1.012E+01	9.975E-02	2.498E-04	2.111E-04	3.908E-05	9.065E-09
1.050E+01	9.975E-02	2.498E-04	2.096E-04	4.051E-05	9.062E-09
1.087E+01	9.975E-02	2.498E-04	2.082E-04	4.194E-05	9.060E-09
1.125E+01	9.975E-02	2.498E-04	2.068E-04	4.335E-05	9.059E-09
1.162E+01	9.975E-02	2.498E-04	2.054E-04	4.476E-05	9.059E-09
1.200E+01	9.975E-02	2.498E-04	2.040E-04	4.616E-05	9.059E-09
1.237E+01	9.975E-02	2.498E-04	2.026E-04	4.754E-05	9.058E-09
1.275E+01	9.975E-02	2.498E-04	2.013E-04	4.892E-05	9.058E-09
1.313E+01	9.975E-02	2.498E-04	1.999E-04	5.029E-05	9.058E-09
1.350E+01	9.975E-02	2.498E-04	1.986E-04	5.165E-05	9.058E-09
1.387E+01	9.975E-02	2.498E-04	1.972E-04	5.300E-05	9.057E-09
1.425E+01	9.975E-02	2.498E-04	1.959E-04	5.434E-05	9.057E-09
1.462E+01	9.975E-02	2.498E-04	1.946E-04	5.567E-05	9.057E-09
1.500E+01	9.975E-02	2.498E-04	1.932E-04	5.699E-05	9.057E-09

TABLE 17

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E-01 ACT= 9.170E-04 Catalase Activity = 1.23×10^{-3} units/ml

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	1.250E-03
3.750E-01	9.984E-02	1.615E-04	1.610E-04	5.521E-07	1.088E-03
7.500E-01	9.968E-02	3.180E-04	3.158E-04	2.182E-06	9.320E-04
1.125E+00	9.953E-02	4.683E-04	4.635E-04	4.843E-06	7.817E-04
1.500E+00	9.939E-02	6.109E-04	6.025E-04	8.484E-06	6.390E-04
1.875E+00	9.926E-02	7.441E-04	7.311E-04	1.304E-05	5.059E-04
2.250E+00	9.913E-02	8.653E-04	8.470E-04	1.843E-05	3.846E-04
2.625E+00	9.903E-02	9.718E-04	9.474E-04	2.456E-05	2.781E-04
3.000E+00	9.894E-02	1.061E-03	1.029E-03	3.131E-05	1.894E-04
3.375E+00	9.887E-02	1.129E-03	1.091E-03	3.855E-05	1.205E-04
3.750E+00	9.882E-02	1.178E-03	1.132E-03	4.614E-05	7.160E-05
4.125E+00	9.879E-02	1.210E-03	1.156E-03	5.396E-05	4.011E-05
4.500E+00	9.877E-02	1.228E-03	1.167E-03	6.188E-05	2.151E-05
4.875E+00	9.876E-02	1.239E-03	1.169E-03	6.985E-05	1.122E-05
5.250E+00	9.876E-02	1.244E-03	1.167E-03	7.782E-05	5.772E-06
5.625E+00	9.875E-02	1.247E-03	1.162E-03	8.576E-05	2.953E-06
6.000E+00	9.875E-02	1.248E-03	1.155E-03	9.366E-05	1.514E-06
6.375E+00	9.875E-02	1.249E-03	1.148E-03	1.015E-04	7.858E-07
6.750E+00	9.875E-02	1.249E-03	1.141E-03	1.093E-04	4.181E-07
7.125E+00	9.875E-02	1.249E-03	1.133E-03	1.171E-04	2.329E-07
7.500E+00	9.875E-02	1.249E-03	1.126E-03	1.248E-04	1.396E-07
7.875E+00	9.875E-02	1.249E-03	1.118E-03	1.324E-04	9.275E-08
8.250E+00	9.875E-02	1.249E-03	1.110E-03	1.400E-04	6.916E-08
8.625E+00	9.875E-02	1.249E-03	1.103E-03	1.476E-04	5.730E-08
9.000E+00	9.875E-02	1.249E-03	1.095E-03	1.551E-04	5.134E-08
9.375E+00	9.875E-02	1.249E-03	1.088E-03	1.625E-04	4.834E-08
9.750E+00	9.875E-02	1.249E-03	1.081E-03	1.699E-04	4.683E-08
1.012E+01	9.875E-02	1.249E-03	1.073E-03	1.773E-04	4.607E-08
1.050E+01	9.875E-02	1.249E-03	1.066E-03	1.845E-04	4.569E-08
1.087E+01	9.875E-02	1.249E-03	1.059E-03	1.918E-04	4.550E-08
1.125E+01	9.875E-02	1.249E-03	1.052E-03	1.990E-04	4.540E-08
1.162E+01	9.875E-02	1.249E-03	1.045E-03	2.061E-04	4.535E-08
1.200E+01	9.875E-02	1.249E-03	1.038E-03	2.132E-04	4.533E-08
1.237E+01	9.875E-02	1.249E-03	1.031E-03	2.203E-04	4.531E-08
1.275E+01	9.875E-02	1.249E-03	1.024E-03	2.273E-04	4.530E-08
1.313E+01	9.875E-02	1.249E-03	1.017E-03	2.343E-04	4.530E-08
1.350E+01	9.875E-02	1.249E-03	1.010E-03	2.412E-04	4.530E-08
1.387E+01	9.875E-02	1.249E-03	1.003E-03	2.480E-04	4.530E-08
1.425E+01	9.875E-02	1.249E-03	9.962E-04	2.548E-04	4.529E-08
1.462E+01	9.875E-02	1.249E-03	9.894E-04	2.616E-04	4.529E-08
1.500E+01	9.875E-02	1.249E-03	9.827E-04	2.683E-04	4.529E-08

TABLE 18

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E-01 ACT= 9.170E-04 Catalase Activity = 1.23×10^{-3} units/ml

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	2.500E-03
3.750E-01	9.982E-02	1.802E-04	1.796E-04	6.140E-07	2.320E-03
7.500E-01	9.964E-02	3.587E-04	3.563E-04	2.443E-06	2.141E-03
1.125E+00	9.946E-02	5.353E-04	5.299E-04	5.467E-06	1.965E-03
1.500E+00	9.929E-02	7.098E-04	7.002E-04	9.663E-06	1.790E-03
1.875E+00	9.912E-02	8.817E-04	8.668E-04	1.501E-05	1.618E-03
2.250E+00	9.895E-02	1.051E-03	1.029E-03	2.148E-05	1.449E-03
2.625E+00	9.878E-02	1.217E-03	1.188E-03	2.904E-05	1.283E-03
3.000E+00	9.862E-02	1.378E-03	1.341E-03	3.767E-05	1.122E-03
3.375E+00	9.846E-02	1.535E-03	1.488E-03	4.732E-05	9.647E-04
3.750E+00	9.831E-02	1.686E-03	1.629E-03	5.795E-05	8.136E-04
4.125E+00	9.817E-02	1.830E-03	1.761E-03	6.951E-05	6.697E-04
4.500E+00	9.803E-02	1.965E-03	1.884E-03	8.195E-05	5.347E-04
4.875E+00	9.791E-02	2.089E-03	1.994E-03	9.518E-05	4.110E-04
5.250E+00	9.780E-02	2.198E-03	2.090E-03	1.091E-04	3.012E-04
5.625E+00	9.771E-02	2.291E-03	2.168E-03	1.236E-04	2.083E-04
6.000E+00	9.763E-02	2.365E-03	2.227E-03	1.386E-04	1.349E-04
6.375E+00	9.758E-02	2.418E-03	2.265E-03	1.540E-04	8.150E-05
6.750E+00	9.755E-02	2.453E-03	2.285E-03	1.695E-04	4.629E-05
7.125E+00	9.752E-02	2.474E-03	2.291E-03	1.851E-04	2.508E-05
7.500E+00	9.751E-02	2.486E-03	2.287E-03	2.007E-04	1.318E-05
7.875E+00	9.751E-02	2.492E-03	2.278E-03	2.163E-04	6.820E-06
8.250E+00	9.750E-02	2.496E-03	2.266E-03	2.318E-04	3.514E-06
8.625E+00	9.750E-02	2.497E-03	2.252E-03	2.472E-04	1.822E-06
9.000E+00	9.750E-02	2.498E-03	2.238E-03	2.625E-04	9.640E-07
9.375E+00	9.750E-02	2.498E-03	2.223E-03	2.777E-04	5.305E-07
9.750E+00	9.750E-02	2.499E-03	2.208E-03	2.928E-04	3.120E-07
1.012E+01	9.750E-02	2.499E-03	2.193E-03	3.078E-04	2.020E-07
1.050E+01	9.750E-02	2.499E-03	2.178E-03	3.227E-04	1.467E-07
1.087E+01	9.750E-02	2.499E-03	2.164E-03	3.375E-04	1.188E-07
1.125E+01	9.750E-02	2.499E-03	2.149E-03	3.522E-04	1.048E-07
1.162E+01	9.750E-02	2.498E-03	2.135E-03	3.668E-04	9.775E-08
1.200E+01	9.750E-02	2.498E-03	2.120E-03	3.814E-04	9.421E-08
1.237E+01	9.750E-02	2.498E-03	2.106E-03	3.958E-04	9.242E-08
1.275E+01	9.750E-02	2.498E-03	2.091E-03	4.101E-04	9.152E-08
1.313E+01	9.750E-02	2.498E-03	2.077E-03	4.243E-04	9.107E-08
1.350E+01	9.750E-02	2.498E-03	2.063E-03	4.384E-04	9.084E-08
1.387E+01	9.750E-02	2.498E-03	2.049E-03	4.524E-04	9.073E-08
1.425E+01	9.750E-02	2.498E-03	2.035E-03	4.664E-04	9.067E-08
1.462E+01	9.750E-02	2.498E-03	2.022E-03	4.802E-04	9.064E-08
1.500E+01	9.750E-02	2.498E-03	2.008E-03	4.939E-04	9.062E-08

TABLE 19

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E-01 ACT= 9.170E-04 Catalase Activity = 1.23×10^{-3} units/ml

T	S	HP	GL	GA	OX
0.	1.000E+00	0.	0.	0.	2.500E-04
3.750E-01	9.999E-01	9.574E-05	9.540E-05	3.441E-07	1.543E-04
7.500E-01	9.998E-01	1.609E-04	1.596E-04	1.230E-06	8.914E-05
1.125E+00	9.998E-01	2.011E-04	1.987E-04	2.464E-06	4.885E-05
1.500E+00	9.998E-01	2.242E-04	2.203E-04	3.301E-06	2.580E-05
1.875E+00	9.998E-01	2.367E-04	2.312E-04	5.445E-06	1.333E-05
2.250E+00	9.998E-01	2.432E-04	2.362E-04	7.041E-06	6.798E-06
2.625E+00	9.998E-01	2.465E-04	2.379E-04	8.659E-06	3.447E-06
3.000E+00	9.998E-01	2.482E-04	2.388E-04	1.028E-05	1.743E-06
3.375E+00	9.998E-01	2.491E-04	2.373E-04	1.190E-05	8.826E-07
3.750E+00	9.998E-01	2.495E-04	2.361E-04	1.352E-05	4.486E-07
4.125E+00	9.998E-01	2.497E-04	2.347E-04	1.512E-05	2.301E-07
4.500E+00	9.998E-01	2.498E-04	2.332E-04	1.672E-05	1.202E-07
4.875E+00	9.997E-01	2.499E-04	2.317E-04	1.830E-05	6.495E-08
5.250E+00	9.997E-01	2.499E-04	2.302E-04	1.988E-05	3.716E-08
5.625E+00	9.997E-01	2.499E-04	2.286E-04	2.144E-05	2.319E-08
6.000E+00	9.997E-01	2.499E-04	2.271E-04	2.300E-05	1.616E-08
6.375E+00	9.997E-01	2.499E-04	2.255E-04	2.454E-05	1.263E-08
6.750E+00	9.997E-01	2.499E-04	2.240E-04	2.607E-05	1.086E-08
7.125E+00	9.997E-01	2.499E-04	2.225E-04	2.760E-05	9.963E-09
7.500E+00	9.997E-01	2.499E-04	2.210E-04	2.911E-05	9.514E-09
7.875E+00	9.997E-01	2.499E-04	2.195E-04	3.061E-05	9.289E-09
8.250E+00	9.997E-01	2.499E-04	2.180E-04	3.210E-05	9.175E-09
8.625E+00	9.997E-01	2.499E-04	2.165E-04	3.358E-05	9.118E-09
9.000E+00	9.997E-01	2.499E-04	2.151E-04	3.506E-05	9.089E-09
9.375E+00	9.997E-01	2.498E-04	2.136E-04	3.652E-05	9.074E-09
9.750E+00	9.997E-01	2.498E-04	2.122E-04	3.797E-05	9.067E-09
1.012E+01	9.997E-01	2.498E-04	2.107E-04	3.941E-05	9.063E-09
1.050E+01	9.997E-01	2.498E-04	2.093E-04	4.084E-05	9.061E-09
1.087E+01	9.997E-01	2.498E-04	2.079E-04	4.227E-05	9.060E-09
1.125E+01	9.997E-01	2.498E-04	2.065E-04	4.368E-05	9.059E-09
1.162E+01	9.997E-01	2.498E-04	2.051E-04	4.508E-05	9.059E-09
1.200E+01	9.997E-01	2.498E-04	2.037E-04	4.648E-05	9.058E-09
1.237E+01	9.997E-01	2.498E-04	2.023E-04	4.786E-05	9.058E-09
1.275E+01	9.997E-01	2.498E-04	2.010E-04	4.924E-05	9.058E-09
1.313E+01	9.997E-01	2.498E-04	1.996E-04	5.060E-05	9.058E-09
1.350E+01	9.997E-01	2.498E-04	1.982E-04	5.196E-05	9.057E-09
1.387E+01	9.997E-01	2.498E-04	1.969E-04	5.331E-05	9.057E-09
1.425E+01	9.997E-01	2.498E-04	1.956E-04	5.464E-05	9.057E-09
1.462E+01	9.997E-01	2.498E-04	1.942E-04	5.597E-05	9.057E-09
1.500E+01	9.997E-01	2.498E-04	1.929E-04	5.729E-05	9.056E-09

TABLE 20

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E-01 ACT= 9.170E-04 Catalase Activity = 1.23×10^{-3} units/ml

T	S	HP	GL	GA	OX
0.	1.000E+00	0.	0.	0.	1.250E-03
3.750E-01	9.998E-01	2.275E-04	2.267E-04	7.815E-07	1.023E-03
7.500E-01	9.996E-01	4.400E-04	4.370E-04	3.055E-06	8.100E-04
1.125E+00	9.994E-01	6.336E-04	6.270E-04	6.696E-06	6.163E-04
1.500E+00	9.992E-01	8.039E-04	7.924E-04	1.155E-05	4.461E-04
1.875E+00	9.991E-01	9.460E-04	9.286E-04	1.744E-05	3.040E-04
2.250E+00	9.989E-01	1.056E-03	1.032E-03	2.414E-05	1.936E-04
2.625E+00	9.989E-01	1.135E-03	1.104E-03	3.144E-05	1.151E-04
3.000E+00	9.988E-01	1.185E-03	1.146E-03	3.913E-05	6.452E-05
3.375E+00	9.988E-01	1.215E-03	1.169E-03	4.703E-05	3.460E-05
3.750E+00	9.988E-01	1.232E-03	1.177E-03	5.504E-05	1.805E-05
4.125E+00	9.988E-01	1.240E-03	1.178E-03	6.307E-05	9.267E-06
4.500E+00	9.988E-01	1.245E-03	1.174E-03	7.109E-05	4.727E-06
4.875E+00	9.988E-01	1.247E-03	1.169E-03	7.909E-05	2.411E-06
5.250E+00	9.988E-01	1.248E-03	1.162E-03	8.703E-05	1.238E-06
5.625E+00	9.988E-01	1.249E-03	1.155E-03	9.493E-05	6.455E-07
6.000E+00	9.987E-01	1.249E-03	1.147E-03	1.028E-04	3.473E-07
6.375E+00	9.987E-01	1.249E-03	1.140E-03	1.106E-04	1.972E-07
6.750E+00	9.987E-01	1.249E-03	1.132E-03	1.183E-04	1.217E-07
7.125E+00	9.987E-01	1.249E-03	1.124E-03	1.260E-04	8.370E-08
7.500E+00	9.987E-01	1.249E-03	1.117E-03	1.337E-04	6.461E-08
7.875E+00	9.987E-01	1.249E-03	1.109E-03	1.413E-04	5.501E-08
8.250E+00	9.987E-01	1.249E-03	1.102E-03	1.488E-04	5.019E-08
8.625E+00	9.987E-01	1.249E-03	1.094E-03	1.563E-04	4.776E-08
9.000E+00	9.987E-01	1.249E-03	1.087E-03	1.637E-04	4.654E-08
9.375E+00	9.987E-01	1.249E-03	1.080E-03	1.711E-04	4.592E-08
9.750E+00	9.987E-01	1.249E-03	1.072E-03	1.784E-04	4.562E-08
1.012E+01	9.987E-01	1.249E-03	1.065E-03	1.857E-04	4.546E-08
1.050E+01	9.987E-01	1.249E-03	1.058E-03	1.930E-04	4.538E-08
1.087E+01	9.987E-01	1.249E-03	1.051E-03	2.002E-04	4.534E-08
1.125E+01	9.987E-01	1.249E-03	1.043E-03	2.073E-04	4.532E-08
1.162E+01	9.987E-01	1.249E-03	1.036E-03	2.144E-04	4.531E-08
1.200E+01	9.987E-01	1.249E-03	1.029E-03	2.214E-04	4.530E-08
1.237E+01	9.987E-01	1.249E-03	1.022E-03	2.284E-04	4.530E-08
1.275E+01	9.987E-01	1.249E-03	1.016E-03	2.354E-04	4.530E-08
1.313E+01	9.987E-01	1.249E-03	1.009E-03	2.423E-04	4.529E-08
1.350E+01	9.987E-01	1.249E-03	1.002E-03	2.491E-04	4.529E-08
1.387E+01	9.987E-01	1.249E-03	9.951E-04	2.559E-04	4.529E-08
1.425E+01	9.987E-01	1.249E-03	9.883E-04	2.627E-04	4.529E-08
1.462E+01	9.987E-01	1.249E-03	9.816E-04	2.694E-04	4.529E-08
1.500E+01	9.987E-01	1.249E-03	9.750E-04	2.761E-04	4.529E-08

TABLE 21

Computer-Generated Concentration Data For The Substituents

In a Closed Glucose Oxidase Reaction System

X= 1.000E-01 ACT= 9.170E-04 Catalase Activity = 1.23×10^{-3} units/ml

T	S	HP	GL	GA	OX
0.	1.000E+00	0.	0.	0.	2.500E-03
3.750E-01	9.997E-01	2.683E-04	2.674E-04	9.157E-07	2.232E-03
7.500E-01	9.995E-01	5.311E-04	5.275E-04	3.631E-06	1.969E-03
1.125E+00	9.992E-01	7.873E-04	7.793E-04	8.092E-06	1.713E-03
1.500E+00	9.990E-01	1.035E-03	1.021E-03	1.424E-05	1.464E-03
1.875E+00	9.987E-01	1.274E-03	1.252E-03	2.200E-05	1.226E-03
2.250E+00	9.985E-01	1.500E-03	1.469E-03	3.128E-05	1.000E-03
2.625E+00	9.983E-01	1.710E-03	1.669E-03	4.199E-05	7.894E-04
3.000E+00	9.981E-01	1.902E-03	1.848E-03	5.400E-05	5.979E-04
3.375E+00	9.979E-01	2.069E-03	2.003E-03	6.714E-05	4.303E-04
3.750E+00	9.978E-01	2.208E-03	2.128E-03	8.125E-05	2.913E-04
4.125E+00	9.977E-01	2.315E-03	2.220E-03	9.609E-05	1.843E-04
4.500E+00	9.976E-01	2.391E-03	2.280E-03	1.115E-04	1.089E-04
4.875E+00	9.976E-01	2.439E-03	2.313E-03	1.271E-04	6.074E-05
5.250E+00	9.975E-01	2.467E-03	2.325E-03	1.430E-04	3.249E-05
5.625E+00	9.975E-01	2.482E-03	2.325E-03	1.588E-04	1.693E-05
6.000E+00	9.975E-01	2.491E-03	2.317E-03	1.746E-04	8.709E-06
6.375E+00	9.975E-01	2.495E-03	2.306E-03	1.904E-04	4.464E-06
6.750E+00	9.975E-01	2.497E-03	2.292E-03	2.061E-04	2.300E-06
7.125E+00	9.975E-01	2.498E-03	2.278E-03	2.217E-04	1.204E-06
7.500E+00	9.975E-01	2.498E-03	2.263E-03	2.372E-04	6.512E-07
7.875E+00	9.975E-01	2.499E-03	2.248E-03	2.525E-04	3.727E-07
8.250E+00	9.975E-01	2.499E-03	2.233E-03	2.678E-04	2.325E-07
8.625E+00	9.975E-01	2.499E-03	2.218E-03	2.830E-04	1.620E-07
9.000E+00	9.975E-01	2.499E-03	2.203E-03	2.981E-04	1.265E-07
9.375E+00	9.975E-01	2.499E-03	2.188E-03	3.130E-04	1.087E-07
9.750E+00	9.975E-01	2.499E-03	2.173E-03	3.279E-04	9.969E-08
1.012E+01	9.975E-01	2.499E-03	2.159E-03	3.427E-04	9.518E-08
1.050E+01	9.975E-01	2.498E-03	2.144E-03	3.574E-04	9.291E-08
1.087E+01	9.975E-01	2.498E-03	2.129E-03	3.719E-04	9.177E-08
1.125E+01	9.975E-01	2.498E-03	2.115E-03	3.864E-04	9.119E-08
1.162E+01	9.975E-01	2.498E-03	2.101E-03	4.008E-04	9.090E-08
1.200E+01	9.975E-01	2.498E-03	2.087E-03	4.151E-04	9.075E-08
1.237E+01	9.975E-01	2.498E-03	2.072E-03	4.292E-04	9.068E-08
1.275E+01	9.975E-01	2.498E-03	2.058E-03	4.433E-04	9.064E-08
1.313E+01	9.975E-01	2.498E-03	2.044E-03	4.573E-04	9.062E-08
1.350E+01	9.975E-01	2.498E-03	2.031E-03	4.712E-04	9.061E-08
1.387E+01	9.975E-01	2.498E-03	2.017E-03	4.850E-04	9.060E-08
1.425E+01	9.975E-01	2.498E-03	2.003E-03	4.987E-04	9.060E-08
1.462E+01	9.975E-01	2.498E-03	1.990E-03	5.123E-04	9.059E-08
1.500E+01	9.975E-01	2.498E-03	1.976E-03	5.259E-04	9.059E-08

TABLE 22

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+00 ACT= 9.170E-03 Catalase Activity = .012 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-02	0.	0.	0.	2.500E-04
7.500E-02	9.934E-03	6.646E-05	6.641E-05	5.462E-08	1.835E-04
1.500E-01	9.873E-03	1.269E-04	1.267E-04	2.125E-07	1.231E-04
2.250E-01	9.822E-03	1.783E-04	1.779E-04	4.614E-07	7.164E-05
3.000E-01	9.784E-03	2.163E-04	2.156E-04	7.832E-07	3.363E-05
3.750E-01	9.762E-03	2.379E-04	2.368E-04	1.153E-06	1.209E-05
4.500E-01	9.753E-03	2.464E-04	2.450E-04	1.545E-06	3.522E-06
5.250E-01	9.751E-03	2.490E-04	2.472E-04	1.946E-06	9.355E-07
6.000E-01	9.750E-03	2.497E-04	2.475E-04	2.348E-06	2.459E-07
6.750E-01	9.750E-03	2.498E-04	2.473E-04	2.749E-06	6.912E-08
7.500E-01	9.750E-03	2.499E-04	2.469E-04	3.151E-06	2.426E-08
8.250E-01	9.750E-03	2.499E-04	2.465E-04	3.552E-06	1.290E-08
9.000E-01	9.750E-03	2.499E-04	2.462E-04	3.952E-06	1.003E-08
9.750E-01	9.750E-03	2.499E-04	2.458E-04	4.351E-06	9.307E-09
1.050E+00	9.750E-03	2.498E-04	2.454E-04	4.750E-06	9.123E-09
1.125E+00	9.750E-03	2.498E-04	2.450E-04	5.149E-06	9.076E-09
1.200E+00	9.750E-03	2.498E-04	2.446E-04	5.546E-06	9.064E-09
1.275E+00	9.750E-03	2.498E-04	2.442E-04	5.943E-06	9.061E-09
1.350E+00	9.750E-03	2.498E-04	2.438E-04	6.340E-06	9.059E-09
1.425E+00	9.750E-03	2.498E-04	2.435E-04	6.736E-06	9.059E-09
1.500E+00	9.750E-03	2.498E-04	2.431E-04	7.131E-06	9.058E-09
1.575E+00	9.750E-03	2.498E-04	2.427E-04	7.525E-06	9.058E-09
1.650E+00	9.750E-03	2.497E-04	2.423E-04	7.919E-06	9.057E-09
1.725E+00	9.750E-03	2.497E-04	2.419E-04	8.313E-06	9.057E-09
1.800E+00	9.750E-03	2.497E-04	2.416E-04	8.706E-06	9.056E-09
1.875E+00	9.750E-03	2.497E-04	2.412E-04	9.098E-06	9.056E-09
1.950E+00	9.750E-03	2.497E-04	2.408E-04	9.489E-06	9.055E-09
2.025E+00	9.750E-03	2.497E-04	2.404E-04	9.880E-06	9.055E-09
2.100E+00	9.750E-03	2.497E-04	2.400E-04	1.027E-05	9.055E-09
2.175E+00	9.750E-03	2.497E-04	2.397E-04	1.066E-05	9.054E-09
2.250E+00	9.750E-03	2.496E-04	2.393E-04	1.105E-05	9.054E-09
2.325E+00	9.750E-03	2.496E-04	2.389E-04	1.144E-05	9.053E-09
2.400E+00	9.750E-03	2.496E-04	2.385E-04	1.183E-05	9.053E-09
2.475E+00	9.750E-03	2.496E-04	2.382E-04	1.221E-05	9.052E-09
2.550E+00	9.750E-03	2.496E-04	2.378E-04	1.260E-05	9.052E-09
2.625E+00	9.750E-03	2.496E-04	2.374E-04	1.298E-05	9.051E-09
2.700E+00	9.750E-03	2.496E-04	2.370E-04	1.337E-05	9.051E-09
2.775E+00	9.750E-03	2.496E-04	2.367E-04	1.376E-05	9.051E-09
2.850E+00	9.750E-03	2.495E-04	2.363E-04	1.414E-05	9.050E-09
2.925E+00	9.750E-03	2.495E-04	2.359E-04	1.452E-05	9.050E-09
3.000E+00	9.750E-03	2.495E-04	2.356E-04	1.491E-05	9.049E-09

TABLE 23

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+00 AGT= 9.170E-03 Catalase Activity = .012 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-02	0.	0.	0.	1.250E-03
7.500E-02	9.919E-03	8.146E-05	8.140E-05	6.625E-08	1.169E-03
1.500E-01	9.838E-03	1.621E-04	1.618E-04	2.639E-07	1.088E-03
2.250E-01	9.758E-03	2.418E-04	2.413E-04	5.915E-07	1.008E-03
3.000E-01	9.679E-03	3.207E-04	3.197E-04	1.047E-06	9.293E-04
3.750E-01	9.601E-03	3.986E-04	3.971E-04	1.630E-06	8.514E-04
4.500E-01	9.524E-03	4.755E-04	4.733E-04	2.337E-06	7.744E-04
5.250E-01	9.448E-03	5.514E-04	5.484E-04	3.167E-06	6.985E-04
6.000E-01	9.374E-03	6.261E-04	6.223E-04	4.118E-06	6.238E-04
6.750E-01	9.300E-03	6.996E-04	6.947E-04	5.188E-06	5.502E-04
7.500E-01	9.228E-03	7.717E-04	7.657E-04	6.374E-06	4.781E-04
8.250E-01	9.157E-03	8.422E-04	8.350E-04	7.675E-06	4.076E-04
9.000E-01	9.089E-03	9.108E-04	9.022E-04	9.086E-06	3.390E-04
9.750E-01	9.022E-03	9.770E-04	9.670E-04	1.060E-05	2.727E-04
1.050E+00	8.959E-03	1.040E-03	1.029E-03	1.223E-05	2.095E-04
1.125E+00	8.900E-03	1.099E-03	1.086E-03	1.394E-05	1.505E-04
1.200E+00	8.847E-03	1.152E-03	1.137E-03	1.575E-05	9.755E-05
1.275E+00	8.803E-03	1.196E-03	1.179E-03	1.763E-05	5.388E-05
1.350E+00	8.773E-03	1.226E-03	1.208E-03	1.957E-05	2.353E-05
1.425E+00	8.757E-03	1.241E-03	1.221E-03	2.155E-05	7.918E-06
1.500E+00	8.752E-03	1.247E-03	1.225E-03	2.354E-05	2.253E-06
1.575E+00	8.750E-03	1.249E-03	1.225E-03	2.553E-05	6.211E-07
1.650E+00	8.749E-03	1.249E-03	1.223E-03	2.752E-05	1.923E-07
1.725E+00	8.749E-03	1.249E-03	1.221E-03	2.950E-05	8.262E-08
1.800E+00	8.749E-03	1.249E-03	1.219E-03	3.148E-05	5.478E-08
1.875E+00	8.749E-03	1.249E-03	1.218E-03	3.346E-05	4.772E-08
1.950E+00	8.749E-03	1.249E-03	1.216E-03	3.544E-05	4.593E-08
2.025E+00	8.749E-03	1.249E-03	1.214E-03	3.741E-05	4.547E-08
2.100E+00	8.749E-03	1.249E-03	1.212E-03	3.938E-05	4.536E-08
2.175E+00	8.749E-03	1.249E-03	1.210E-03	4.135E-05	4.532E-08
2.250E+00	8.749E-03	1.249E-03	1.208E-03	4.331E-05	4.531E-08
2.325E+00	8.749E-03	1.249E-03	1.206E-03	4.527E-05	4.531E-08
2.400E+00	8.749E-03	1.248E-03	1.204E-03	4.723E-05	4.531E-08
2.475E+00	8.749E-03	1.248E-03	1.202E-03	4.919E-05	4.531E-08
2.550E+00	8.748E-03	1.248E-03	1.200E-03	5.114E-05	4.530E-08
2.625E+00	8.748E-03	1.248E-03	1.199E-03	5.309E-05	4.530E-08
2.700E+00	8.748E-03	1.248E-03	1.197E-03	5.503E-05	4.530E-08
2.775E+00	8.748E-03	1.248E-03	1.195E-03	5.697E-05	4.530E-08
2.850E+00	8.748E-03	1.248E-03	1.193E-03	5.891E-05	4.529E-08
2.925E+00	8.748E-03	1.248E-03	1.191E-03	6.085E-05	4.529E-08
3.000E+00	8.748E-03	1.248E-03	1.189E-03	6.278E-05	4.529E-08

TABLE 24

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+00 ACT= 9.170E-03 Catalase = .012 units/ml Activity

T	S	HP	GL	GA	OX
0.	1.000E-02	0.	0.	0.	2.500E-03
7.500E-02	9.916E-03	8.353E-05	8.347E-05	6.791E-08	2.416E-03
1.500E-01	9.834E-03	1.664E-04	1.661E-04	2.708E-07	2.334E-03
2.250E-01	9.751E-03	2.486E-04	2.480E-04	6.073E-07	2.251E-03
3.000E-01	9.670E-03	3.301E-04	3.291E-04	1.076E-06	2.170E-03
3.750E-01	9.589E-03	4.109E-04	4.093E-04	1.676E-06	2.089E-03
4.500E-01	9.509E-03	4.911E-04	4.888E-04	2.406E-06	2.009E-03
5.250E-01	9.429E-03	5.706E-04	5.675E-04	3.264E-06	1.929E-03
6.000E-01	9.350E-03	6.494E-04	6.454E-04	4.249E-06	1.850E-03
6.750E-01	9.272E-03	7.275E-04	7.225E-04	5.360E-06	1.772E-03
7.500E-01	9.195E-03	8.050E-04	7.988E-04	6.596E-06	1.695E-03
8.250E-01	9.118E-03	8.818E-04	8.743E-04	7.955E-06	1.618E-03
9.000E-01	9.042E-03	9.579E-04	9.490E-04	9.436E-06	1.542E-03
9.750E-01	8.966E-03	1.033E-03	1.023E-03	1.104E-05	1.466E-03
1.050E+00	8.891E-03	1.108E-03	1.096E-03	1.276E-05	1.392E-03
1.125E+00	8.817E-03	1.182E-03	1.168E-03	1.460E-05	1.317E-03
1.200E+00	8.744E-03	1.255E-03	1.240E-03	1.655E-05	1.244E-03
1.275E+00	8.671E-03	1.328E-03	1.311E-03	1.863E-05	1.171E-03
1.350E+00	8.599E-03	1.400E-03	1.380E-03	2.081E-05	1.099E-03
1.425E+00	8.527E-03	1.471E-03	1.449E-03	2.311E-05	1.028E-03
1.500E+00	8.457E-03	1.541E-03	1.518E-03	2.552E-05	9.577E-04
1.575E+00	8.387E-03	1.611E-03	1.585E-03	2.804E-05	8.880E-04
1.650E+00	8.318E-03	1.680E-03	1.651E-03	3.067E-05	8.191E-04
1.725E+00	8.250E-03	1.748E-03	1.717E-03	3.341E-05	7.510E-04
1.800E+00	8.183E-03	1.815E-03	1.781E-03	3.625E-05	6.838E-04
1.875E+00	8.116E-03	1.881E-03	1.845E-03	3.919E-05	6.175E-04
1.950E+00	8.051E-03	1.947E-03	1.907E-03	4.224E-05	5.522E-04
2.025E+00	7.987E-03	2.011E-03	1.968E-03	4.539E-05	4.879E-04
2.100E+00	7.923E-03	2.074E-03	2.028E-03	4.863E-05	4.249E-04
2.175E+00	7.862E-03	2.135E-03	2.086E-03	5.197E-05	3.633E-04
2.250E+00	7.802E-03	2.195E-03	2.143E-03	5.541E-05	3.033E-04
2.325E+00	7.743E-03	2.253E-03	2.198E-03	5.894E-05	2.453E-04
2.400E+00	7.688E-03	2.308E-03	2.250E-03	6.255E-05	1.899E-04
2.475E+00	7.636E-03	2.360E-03	2.298E-03	6.624E-05	1.379E-04
2.550E+00	7.589E-03	2.407E-03	2.341E-03	7.001E-05	9.103E-05
2.625E+00	7.549E-03	2.446E-03	2.377E-03	7.384E-05	5.172E-05
2.700E+00	7.521E-03	2.474E-03	2.401E-03	7.773E-05	2.350E-05
2.775E+00	7.506E-03	2.489E-03	2.413E-03	8.164E-05	8.233E-06
2.850E+00	7.500E-03	2.495E-03	2.415E-03	8.556E-05	2.422E-06
2.925E+00	7.498E-03	2.496E-03	2.413E-03	8.948E-05	7.043E-07
3.000E+00	7.497E-03	2.497E-03	2.409E-03	9.340E-05	2.480E-07

TABLE 25

Computer-Generated Concentration Data For The Substituents in a Closed Glucose Oxidase Reaction System

X= 1.000E+00 ACT= 9.170E-03 Catalase Activity = .012 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	2.500E-04
7.500E-02	9.985E-02	1.461E-04	1.460E-04	1.296E-07	1.039E-04
1.500E-01	9.976E-02	2.166E-04	2.162E-04	4.326E-07	3.337E-05
2.250E-01	9.976E-02	2.408E-04	2.401E-04	8.071E-07	9.163E-06
3.000E-01	9.975E-02	2.476E-04	2.465E-04	1.203E-06	2.377E-06
3.750E-01	9.975E-02	2.493E-04	2.478E-04	1.605E-06	6.110E-07
4.500E-01	9.975E-02	2.498E-04	2.479E-04	2.008E-06	1.614E-07
5.250E-01	9.975E-02	2.499E-04	2.476E-04	2.410E-06	4.758E-08
6.000E-01	9.975E-02	2.499E-04	2.473E-04	2.812E-06	1.880E-08
6.750E-01	9.975E-02	2.499E-04	2.469E-04	3.214E-06	1.152E-08
7.500E-01	9.975E-02	2.499E-04	2.465E-04	3.615E-06	3.683E-09
8.250E-01	9.975E-02	2.499E-04	2.461E-04	4.015E-06	9.218E-09
9.000E-01	9.975E-02	2.499E-04	2.457E-04	4.414E-06	9.100E-09
9.750E-01	9.975E-02	2.498E-04	2.453E-04	4.813E-06	9.070E-09
1.050E+00	9.975E-02	2.498E-04	2.449E-04	5.211E-06	9.062E-09
1.125E+00	9.975E-02	2.498E-04	2.446E-04	5.609E-06	9.059E-09
1.200E+00	9.975E-02	2.498E-04	2.442E-04	6.006E-06	9.059E-09
1.275E+00	9.975E-02	2.498E-04	2.438E-04	6.402E-06	9.058E-09
1.350E+00	9.975E-02	2.498E-04	2.434E-04	6.798E-06	9.058E-09
1.425E+00	9.975E-02	2.498E-04	2.430E-04	7.193E-06	9.057E-09
1.500E+00	9.975E-02	2.498E-04	2.426E-04	7.587E-06	9.057E-09
1.575E+00	9.975E-02	2.497E-04	2.423E-04	7.981E-06	9.056E-09
1.650E+00	9.975E-02	2.497E-04	2.419E-04	8.374E-06	9.056E-09
1.725E+00	9.975E-02	2.497E-04	2.415E-04	8.767E-06	9.055E-09
1.800E+00	9.975E-02	2.497E-04	2.411E-04	9.159E-06	9.055E-09
1.875E+00	9.975E-02	2.497E-04	2.407E-04	9.550E-06	9.054E-09
1.950E+00	9.975E-02	2.497E-04	2.404E-04	9.941E-06	9.054E-09
2.025E+00	9.975E-02	2.497E-04	2.400E-04	1.033E-05	9.053E-09
2.100E+00	9.975E-02	2.497E-04	2.396E-04	1.072E-05	9.053E-09
2.175E+00	9.975E-02	2.496E-04	2.392E-04	1.111E-05	9.053E-09
2.250E+00	9.975E-02	2.496E-04	2.389E-04	1.150E-05	9.052E-09
2.325E+00	9.975E-02	2.496E-04	2.385E-04	1.189E-05	9.052E-09
2.400E+00	9.975E-02	2.496E-04	2.381E-04	1.227E-05	9.051E-09
2.475E+00	9.975E-02	2.496E-04	2.377E-04	1.266E-05	9.051E-09
2.550E+00	9.975E-02	2.496E-04	2.374E-04	1.305E-05	9.050E-09
2.625E+00	9.975E-02	2.496E-04	2.370E-04	1.343E-05	9.050E-09
2.700E+00	9.975E-02	2.496E-04	2.366E-04	1.382E-05	9.049E-09
2.775E+00	9.975E-02	2.495E-04	2.362E-04	1.420E-05	9.049E-09
2.850E+00	9.975E-02	2.495E-04	2.359E-04	1.458E-05	9.048E-09
2.925E+00	9.975E-02	2.495E-04	2.355E-04	1.497E-05	9.048E-09
3.000E+00	9.975E-02	2.495E-04	2.351E-04	1.535E-05	9.048E-09

TABLE 26

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+00 ACT= 9.170E-03 Catalase Activity = .012 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	1.250E-03
7.500E-02	9.968E-02	3.180E-04	3.177E-04	2.609E-07	9.320E-04
1.500E-01	9.939E-02	6.109E-04	6.100E-04	1.019E-06	6.390E-04
2.250E-01	9.913E-02	8.653E-04	8.632E-04	2.222E-06	3.846E-04
3.000E-01	9.894E-02	1.061E-03	1.057E-03	3.791E-06	1.894E-04
3.750E-01	9.882E-02	1.178E-03	1.173E-03	5.613E-06	7.160E-05
4.500E-01	9.877E-02	1.228E-03	1.221E-03	7.565E-06	2.151E-05
5.250E-01	9.876E-02	1.244E-03	1.235E-03	9.562E-06	5.772E-06
6.000E-01	9.875E-02	1.248E-03	1.237E-03	1.157E-05	1.514E-06
6.750E-01	9.875E-02	1.249E-03	1.236E-03	1.358E-05	4.181E-07
7.500E-01	9.875E-02	1.249E-03	1.235E-03	1.559E-05	1.396E-07
8.250E-01	9.875E-02	1.249E-03	1.233E-03	1.759E-05	6.916E-08
9.000E-01	9.875E-02	1.249E-03	1.231E-03	1.959E-05	5.134E-08
9.750E-01	9.875E-02	1.249E-03	1.229E-03	2.159E-05	4.683E-08
1.050E+00	9.875E-02	1.249E-03	1.227E-03	2.359E-05	4.569E-08
1.125E+00	9.875E-02	1.249E-03	1.225E-03	2.558E-05	4.540E-08
1.200E+00	9.875E-02	1.249E-03	1.223E-03	2.757E-05	4.533E-08
1.275E+00	9.875E-02	1.249E-03	1.221E-03	2.955E-05	4.530E-08
1.350E+00	9.875E-02	1.249E-03	1.219E-03	3.154E-05	4.530E-08
1.425E+00	9.875E-02	1.249E-03	1.217E-03	3.351E-05	4.529E-08
1.500E+00	9.875E-02	1.249E-03	1.216E-03	3.549E-05	4.529E-08
1.575E+00	9.875E-02	1.249E-03	1.214E-03	3.746E-05	4.529E-08
1.650E+00	9.875E-02	1.249E-03	1.212E-03	3.943E-05	4.529E-08
1.725E+00	9.875E-02	1.249E-03	1.210E-03	4.140E-05	4.528E-08
1.800E+00	9.875E-02	1.249E-03	1.208E-03	4.336E-05	4.528E-08
1.875E+00	9.875E-02	1.249E-03	1.206E-03	4.533E-05	4.528E-08
1.950E+00	9.875E-02	1.248E-03	1.204E-03	4.728E-05	4.528E-08
2.025E+00	9.875E-02	1.248E-03	1.202E-03	4.924E-05	4.528E-08
2.100E+00	9.875E-02	1.248E-03	1.200E-03	5.119E-05	4.527E-08
2.175E+00	9.875E-02	1.248E-03	1.198E-03	5.314E-05	4.527E-08
2.250E+00	9.875E-02	1.248E-03	1.197E-03	5.508E-05	4.527E-08
2.325E+00	9.875E-02	1.248E-03	1.195E-03	5.703E-05	4.527E-08
2.400E+00	9.875E-02	1.248E-03	1.193E-03	5.896E-05	4.526E-08
2.475E+00	9.875E-02	1.248E-03	1.191E-03	6.090E-05	4.526E-08
2.550E+00	9.875E-02	1.248E-03	1.189E-03	6.283E-05	4.526E-08
2.625E+00	9.875E-02	1.248E-03	1.187E-03	6.476E-05	4.526E-08
2.700E+00	9.875E-02	1.248E-03	1.185E-03	6.669E-05	4.526E-08
2.775E+00	9.875E-02	1.248E-03	1.184E-03	6.862E-05	4.525E-08
2.850E+00	9.875E-02	1.248E-03	1.182E-03	7.054E-05	4.525E-08
2.925E+00	9.875E-02	1.248E-03	1.180E-03	7.245E-05	4.525E-08
3.000E+00	9.875E-02	1.248E-03	1.178E-03	7.437E-05	4.525E-08

TABLE 27

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+00 ACT= 9.170E-03 Catalase = .012 units/ml Activity

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	2.500E-03
7.500E-02	9.964E-02	3.587E-04	3.584E-04	2.921E-07	2.141E-03
1.500E-01	9.929E-02	7.098E-04	7.087E-04	1.160E-06	1.790E-03
2.250E-01	9.895E-02	1.051E-03	1.048E-03	2.589E-06	1.449E-03
3.000E-01	9.862E-02	1.378E-03	1.374E-03	4.559E-06	1.122E-03
3.750E-01	9.831E-02	1.686E-03	1.680E-03	7.042E-06	8.136E-04
4.500E-01	9.803E-02	1.965E-03	1.956E-03	1.000E-05	5.347E-04
5.250E-01	9.780E-02	2.198E-03	2.186E-03	1.337E-05	3.012E-04
6.000E-01	9.763E-02	2.365E-03	2.349E-03	1.706E-05	1.349E-04
6.750E-01	9.755E-02	2.453E-03	2.433E-03	2.096E-05	4.629E-05
7.500E-01	9.751E-02	2.486E-03	2.463E-03	2.494E-05	1.318E-05
8.250E-01	9.750E-02	2.496E-03	2.468E-03	2.895E-05	3.514E-06
9.000E-01	9.750E-02	2.498E-03	2.467E-03	3.296E-05	9.640E-07
9.750E-01	9.750E-02	2.499E-03	2.464E-03	3.696E-05	3.120E-07
1.050E+00	9.750E-02	2.499E-03	2.460E-03	4.096E-05	1.467E-07
1.125E+00	9.750E-02	2.499E-03	2.456E-03	4.496E-05	1.048E-07
1.200E+00	9.750E-02	2.498E-03	2.452E-03	4.894E-05	9.421E-08
1.275E+00	9.750E-02	2.498E-03	2.449E-03	5.292E-05	9.152E-08
1.350E+00	9.750E-02	2.498E-03	2.445E-03	5.690E-05	9.084E-08
1.425E+00	9.750E-02	2.498E-03	2.441E-03	6.087E-05	9.067E-08
1.500E+00	9.750E-02	2.498E-03	2.437E-03	6.483E-05	9.062E-08
1.575E+00	9.750E-02	2.498E-03	2.433E-03	6.878E-05	9.060E-08
1.650E+00	9.750E-02	2.498E-03	2.429E-03	7.273E-05	9.060E-08
1.725E+00	9.750E-02	2.498E-03	2.426E-03	7.668E-05	9.059E-08
1.800E+00	9.750E-02	2.497E-03	2.422E-03	8.062E-05	9.059E-08
1.875E+00	9.750E-02	2.497E-03	2.418E-03	8.455E-05	9.058E-08
1.950E+00	9.750E-02	2.497E-03	2.414E-03	8.847E-05	9.058E-08
2.025E+00	9.750E-02	2.497E-03	2.410E-03	9.239E-05	9.057E-08
2.100E+00	9.750E-02	2.497E-03	2.407E-03	9.630E-05	9.057E-08
2.175E+00	9.750E-02	2.497E-03	2.403E-03	1.002E-04	9.056E-08
2.250E+00	9.750E-02	2.497E-03	2.399E-03	1.041E-04	9.056E-08
2.325E+00	9.750E-02	2.497E-03	2.395E-03	1.080E-04	9.055E-08
2.400E+00	9.750E-02	2.496E-03	2.392E-03	1.119E-04	9.055E-08
2.475E+00	9.750E-02	2.496E-03	2.388E-03	1.158E-04	9.055E-08
2.550E+00	9.750E-02	2.496E-03	2.384E-03	1.196E-04	9.054E-08
2.625E+00	9.750E-02	2.496E-03	2.380E-03	1.235E-04	9.054E-08
2.700E+00	9.750E-02	2.496E-03	2.377E-03	1.274E-04	9.053E-08
2.775E+00	9.750E-02	2.496E-03	2.373E-03	1.312E-04	9.053E-08
2.850E+00	9.750E-02	2.496E-03	2.369E-03	1.351E-04	9.052E-08
2.925E+00	9.750E-02	2.496E-03	2.365E-03	1.389E-04	9.052E-08
3.000E+00	9.750E-02	2.495E-03	2.362E-03	1.428E-04	9.051E-08

TABLE 28

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+00 ACT= 9.170E-03 Catalase = .012 units/ml Activity

T	S	HP	GL	GA	OX
0.	1.000E+00	0.	0.	0.	2.500E-04
7.500E-02	9.998E-01	1.609E-04	1.607E-04	1.471E-07	8.914E-05
1.500E-01	9.998E-01	2.242E-04	2.237E-04	4.688E-07	2.580E-05
2.250E-01	9.998E-01	2.432E-04	2.424E-04	8.506E-07	6.798E-06
3.000E-01	9.998E-01	2.482E-04	2.470E-04	1.249E-06	1.743E-06
3.750E-01	9.998E-01	2.495E-04	2.480E-04	1.651E-06	4.486E-07
4.500E-01	9.998E-01	2.498E-04	2.479E-04	2.054E-06	1.202E-07
5.250E-01	9.997E-01	2.499E-04	2.476E-04	2.457E-06	3.716E-08
6.000E-01	9.997E-01	2.499E-04	2.472E-04	2.858E-06	1.616E-08
6.750E-01	9.997E-01	2.499E-04	2.468E-04	3.260E-06	1.086E-08
7.500E-01	9.997E-01	2.499E-04	2.464E-04	3.660E-06	9.514E-09
8.250E-01	9.997E-01	2.499E-04	2.461E-04	4.060E-06	9.175E-09
9.000E-01	9.997E-01	2.499E-04	2.457E-04	4.460E-06	9.089E-09
9.750E-01	9.997E-01	2.498E-04	2.453E-04	4.859E-06	9.067E-09
1.050E+00	9.997E-01	2.498E-04	2.449E-04	5.257E-06	9.061E-09
1.125E+00	9.997E-01	2.498E-04	2.445E-04	5.654E-06	9.059E-09
1.200E+00	9.997E-01	2.498E-04	2.441E-04	6.051E-06	9.058E-09
1.275E+00	9.997E-01	2.498E-04	2.437E-04	6.448E-06	9.058E-09
1.350E+00	9.997E-01	2.498E-04	2.434E-04	6.843E-06	9.057E-09
1.425E+00	9.997E-01	2.498E-04	2.430E-04	7.238E-06	9.057E-09
1.500E+00	9.997E-01	2.498E-04	2.426E-04	7.633E-06	9.056E-09
1.575E+00	9.997E-01	2.497E-04	2.422E-04	8.026E-06	9.056E-09
1.650E+00	9.997E-01	2.497E-04	2.418E-04	8.420E-06	9.056E-09
1.725E+00	9.997E-01	2.497E-04	2.415E-04	8.812E-06	9.055E-09
1.800E+00	9.997E-01	2.497E-04	2.411E-04	9.204E-06	9.055E-09
1.875E+00	9.997E-01	2.497E-04	2.407E-04	9.595E-06	9.054E-09
1.950E+00	9.997E-01	2.497E-04	2.403E-04	9.986E-06	9.054E-09
2.025E+00	9.997E-01	2.497E-04	2.399E-04	1.038E-05	9.053E-09
2.100E+00	9.997E-01	2.497E-04	2.396E-04	1.077E-05	9.053E-09
2.175E+00	9.997E-01	2.496E-04	2.392E-04	1.115E-05	9.052E-09
2.250E+00	9.997E-01	2.496E-04	2.388E-04	1.154E-05	9.052E-09
2.325E+00	9.997E-01	2.496E-04	2.384E-04	1.193E-05	9.051E-09
2.400E+00	9.997E-01	2.496E-04	2.381E-04	1.232E-05	9.051E-09
2.475E+00	9.997E-01	2.496E-04	2.377E-04	1.270E-05	9.051E-09
2.550E+00	9.997E-01	2.496E-04	2.373E-04	1.309E-05	9.050E-09
2.625E+00	9.997E-01	2.496E-04	2.369E-04	1.347E-05	9.050E-09
2.700E+00	9.997E-01	2.496E-04	2.366E-04	1.386E-05	9.049E-09
2.775E+00	9.997E-01	2.496E-04	2.362E-04	1.424E-05	9.049E-09
2.850E+00	9.997E-01	2.495E-04	2.358E-04	1.463E-05	9.048E-09
2.925E+00	9.997E-01	2.495E-04	2.355E-04	1.501E-05	9.048E-09
3.000E+00	9.997E-01	2.496E-04	2.351E-04	1.539E-05	9.047E-09

TABLE 29

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

Y= 1.000E+00 ACT= 9.170E-03 Catalase Activity = .012 units/ml

T	S	HP	GL	GA	OX
0.	1.000E+00	0.	0.	0.	1.250E-03
7.500E-02	9.996E-01	4.400E-04	4.396E-04	3.653E-07	8.100E-04
1.500E-01	9.992E-01	8.039E-04	8.026E-04	1.387E-06	4.461E-04
2.250E-01	9.989E-01	1.056E-03	1.054E-03	2.912E-06	1.936E-04
3.000E-01	9.988E-01	1.185E-03	1.181E-03	4.742E-06	6.452E-05
3.750E-01	9.988E-01	1.232E-03	1.225E-03	6.704E-06	1.805E-05
4.500E-01	9.988E-01	1.245E-03	1.237E-03	8.706E-06	4.727E-06
5.250E-01	9.988E-01	1.248E-03	1.238E-03	1.072E-05	1.238E-06
6.000E-01	9.987E-01	1.249E-03	1.237E-03	1.273E-05	3.473E-07
6.750E-01	9.987E-01	1.249E-03	1.236E-03	1.474E-05	1.217E-07
7.500E-01	9.987E-01	1.249E-03	1.234E-03	1.674E-05	6.461E-08
8.250E-01	9.987E-01	1.249E-03	1.232E-03	1.875E-05	5.019E-08
9.000E-01	9.987E-01	1.249E-03	1.230E-03	2.074E-05	4.654E-08
9.750E-01	9.987E-01	1.249E-03	1.228E-03	2.274E-05	4.562E-08
1.050E+00	9.987E-01	1.249E-03	1.226E-03	2.473E-05	4.538E-08
1.125E+00	9.987E-01	1.249E-03	1.224E-03	2.672E-05	4.532E-08
1.200E+00	9.987E-01	1.249E-03	1.222E-03	2.871E-05	4.530E-08
1.275E+00	9.987E-01	1.249E-03	1.220E-03	3.069E-05	4.530E-08
1.350E+00	9.987E-01	1.249E-03	1.218E-03	3.268E-05	4.529E-08
1.425E+00	9.987E-01	1.249E-03	1.216E-03	3.465E-05	4.529E-08
1.500E+00	9.987E-01	1.249E-03	1.214E-03	3.663E-05	4.529E-08
1.575E+00	9.987E-01	1.249E-03	1.213E-03	3.860E-05	4.529E-08
1.650E+00	9.987E-01	1.249E-03	1.211E-03	4.057E-05	4.528E-08
1.725E+00	9.987E-01	1.249E-03	1.209E-03	4.253E-05	4.528E-08
1.800E+00	9.987E-01	1.249E-03	1.207E-03	4.449E-05	4.528E-08
1.875E+00	9.987E-01	1.249E-03	1.205E-03	4.645E-05	4.528E-08
1.950E+00	9.987E-01	1.248E-03	1.203E-03	4.841E-05	4.527E-08
2.025E+00	9.987E-01	1.248E-03	1.201E-03	5.036E-05	4.527E-08
2.100E+00	9.987E-01	1.248E-03	1.199E-03	5.231E-05	4.527E-08
2.175E+00	9.987E-01	1.248E-03	1.197E-03	5.426E-05	4.527E-08
2.250E+00	9.987E-01	1.248E-03	1.196E-03	5.620E-05	4.526E-08
2.325E+00	9.987E-01	1.248E-03	1.194E-03	5.814E-05	4.526E-08
2.400E+00	9.987E-01	1.248E-03	1.192E-03	6.008E-05	4.526E-08
2.475E+00	9.987E-01	1.248E-03	1.190E-03	6.201E-05	4.526E-08
2.550E+00	9.987E-01	1.248E-03	1.188E-03	6.395E-05	4.526E-08
2.625E+00	9.987E-01	1.248E-03	1.186E-03	6.587E-05	4.525E-08
2.700E+00	9.987E-01	1.248E-03	1.184E-03	6.780E-05	4.525E-08
2.775E+00	9.987E-01	1.248E-03	1.182E-03	6.972E-05	4.525E-08
2.850E+00	9.987E-01	1.248E-03	1.181E-03	7.164E-05	4.525E-08
2.925E+00	9.987E-01	1.248E-03	1.179E-03	7.356E-05	4.524E-08
3.000E+00	9.987E-01	1.248E-03	1.177E-03	7.547E-05	4.524E-08

TABLE 30

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+00 AGT= 9.170E-03 Catalase Activity = .012 units/ml

T	S	HP	GL	GA	OX
0.	1.000E+00	0.	0.	0.	2.500E-03
7.500E-02	9.995E-01	5.311E-04	5.307E-04	4.342E-07	1.969E-03
1.500E-01	9.990E-01	1.035E-03	1.034E-03	1.709E-06	1.464E-03
2.250E-01	9.985E-01	1.500E-03	1.496E-03	3.771E-06	1.000E-03
3.000E-01	9.981E-01	1.902E-03	1.896E-03	6.537E-06	5.979E-04
3.750E-01	9.978E-01	2.208E-03	2.199E-03	9.879E-06	2.913E-04
4.500E-01	9.976E-01	2.391E-03	2.378E-03	1.361E-05	1.089E-04
5.250E-01	9.975E-01	2.467E-03	2.451E-03	1.755E-05	3.249E-05
6.000E-01	9.975E-01	2.491E-03	2.470E-03	2.155E-05	8.709E-06
6.750E-01	9.975E-01	2.497E-03	2.473E-03	2.556E-05	2.300E-06
7.500E-01	9.975E-01	2.498E-03	2.471E-03	2.958E-05	6.512E-07
8.250E-01	9.975E-01	2.499E-03	2.467E-03	3.359E-05	2.325E-07
9.000E-01	9.975E-01	2.499E-03	2.463E-03	3.759E-05	1.265E-07
9.750E-01	9.975E-01	2.499E-03	2.460E-03	4.159E-05	9.969E-08
1.050E+00	9.975E-01	2.498E-03	2.456E-03	4.559E-05	9.291E-08
1.125E+00	9.975E-01	2.498E-03	2.452E-03	4.957E-05	9.119E-08
1.200E+00	9.975E-01	2.498E-03	2.448E-03	5.355E-05	9.075E-08
1.275E+00	9.975E-01	2.498E-03	2.444E-03	5.753E-05	9.064E-08
1.350E+00	9.975E-01	2.498E-03	2.440E-03	6.149E-05	9.061E-08
1.425E+00	9.975E-01	2.498E-03	2.436E-03	6.545E-05	9.060E-08
1.500E+00	9.975E-01	2.498E-03	2.433E-03	6.941E-05	9.059E-08
1.575E+00	9.975E-01	2.498E-03	2.429E-03	7.336E-05	9.058E-08
1.650E+00	9.975E-01	2.498E-03	2.425E-03	7.730E-05	9.058E-08
1.725E+00	9.975E-01	2.497E-03	2.421E-03	8.124E-05	9.057E-08
1.800E+00	9.975E-01	2.497E-03	2.417E-03	8.517E-05	9.057E-08
1.875E+00	9.975E-01	2.497E-03	2.414E-03	8.909E-05	9.057E-08
1.950E+00	9.975E-01	2.497E-03	2.410E-03	9.301E-05	9.056E-08
2.025E+00	9.975E-01	2.497E-03	2.406E-03	9.692E-05	9.056E-08
2.100E+00	9.975E-01	2.497E-03	2.402E-03	1.008E-04	9.055E-08
2.175E+00	9.975E-01	2.497E-03	2.398E-03	1.047E-04	9.055E-08
2.250E+00	9.975E-01	2.497E-03	2.395E-03	1.086E-04	9.054E-08
2.325E+00	9.975E-01	2.496E-03	2.391E-03	1.125E-04	9.054E-08
2.400E+00	9.975E-01	2.496E-03	2.387E-03	1.164E-04	9.053E-08
2.475E+00	9.975E-01	2.496E-03	2.383E-03	1.203E-04	9.053E-08
2.550E+00	9.975E-01	2.496E-03	2.380E-03	1.241E-04	9.052E-08
2.625E+00	9.975E-01	2.496E-03	2.376E-03	1.280E-04	9.052E-08
2.700E+00	9.975E-01	2.496E-03	2.372E-03	1.319E-04	9.052E-08
2.775E+00	9.975E-01	2.496E-03	2.368E-03	1.357E-04	9.051E-08
2.850E+00	9.975E-01	2.496E-03	2.365E-03	1.395E-04	9.051E-08
2.925E+00	9.975E-01	2.495E-03	2.361E-03	1.434E-04	9.050E-08
3.000E+00	9.975E-01	2.495E-03	2.357E-03	1.472E-04	9.050E-08

TABLE 31

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+01 AGT= 9.170E-02 Catalase Activity = .123 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-02	0.	0.	0.	2.500E-04
1.250E-03	9.989E-03	1.140E-05	1.140E-05	4.027E-10	2.386E-04
2.500E-03	9.977E-03	2.269E-05	2.269E-05	1.605E-09	2.273E-04
3.750E-03	9.966E-03	3.384E-05	3.384E-05	3.598E-09	2.162E-04
5.000E-03	9.955E-03	4.486E-05	4.486E-05	6.373E-09	2.051E-04
6.250E-03	9.944E-03	5.574E-05	5.573E-05	9.920E-09	1.943E-04
7.500E-03	9.934E-03	6.646E-05	6.645E-05	1.423E-08	1.835E-04
8.750E-03	9.923E-03	7.701E-05	7.700E-05	1.928E-08	1.730E-04
1.000E-02	9.913E-03	8.740E-05	8.738E-05	2.508E-08	1.626E-04
1.125E-02	9.902E-03	9.759E-05	9.757E-05	3.160E-08	1.524E-04
1.250E-02	9.892E-03	1.076E-04	1.076E-04	3.883E-08	1.424E-04
1.375E-02	9.883E-03	1.174E-04	1.173E-04	4.676E-08	1.326E-04
1.500E-02	9.873E-03	1.269E-04	1.269E-04	5.537E-08	1.231E-04
1.625E-02	9.864E-03	1.362E-04	1.362E-04	6.464E-08	1.138E-04
1.750E-02	9.855E-03	1.453E-04	1.452E-04	7.457E-08	1.047E-04
1.875E-02	9.846E-03	1.540E-04	1.539E-04	8.511E-08	9.598E-05
2.000E-02	9.838E-03	1.625E-04	1.624E-04	9.626E-08	8.753E-05
2.125E-02	9.829E-03	1.706E-04	1.705E-04	1.080E-07	7.941E-05
2.250E-02	9.822E-03	1.783E-04	1.783E-04	1.203E-07	7.164E-05
2.375E-02	9.814E-03	1.857E-04	1.856E-04	1.331E-07	6.424E-05
2.500E-02	9.807E-03	1.927E-04	1.926E-04	1.465E-07	5.725E-05
2.625E-02	9.801E-03	1.993E-04	1.992E-04	1.603E-07	5.067E-05
2.750E-02	9.795E-03	2.055E-04	2.053E-04	1.745E-07	4.453E-05
2.875E-02	9.789E-03	2.111E-04	2.110E-04	1.892E-07	3.884E-05
3.000E-02	9.784E-03	2.163E-04	2.162E-04	2.043E-07	3.363E-05
3.125E-02	9.779E-03	2.211E-04	2.209E-04	2.197E-07	2.889E-05
3.250E-02	9.775E-03	2.253E-04	2.252E-04	2.354E-07	2.463E-05
3.375E-02	9.771E-03	2.291E-04	2.289E-04	2.514E-07	2.084E-05
3.500E-02	9.767E-03	2.325E-04	2.323E-04	2.677E-07	1.750E-05
3.625E-02	9.765E-03	2.354E-04	2.352E-04	2.842E-07	1.459E-05
3.750E-02	9.762E-03	2.379E-04	2.376E-04	3.008E-07	1.209E-05
3.875E-02	9.760E-03	2.400E-04	2.398E-04	3.177E-07	9.955E-06
4.000E-02	9.758E-03	2.418E-04	2.416E-04	3.346E-07	8.154E-06
4.125E-02	9.757E-03	2.433E-04	2.430E-04	3.517E-07	6.648E-06
4.250E-02	9.755E-03	2.446E-04	2.443E-04	3.689E-07	5.397E-06
4.375E-02	9.754E-03	2.456E-04	2.453E-04	3.862E-07	4.366E-06
4.500E-02	9.753E-03	2.464E-04	2.461E-04	4.035E-07	3.522E-06
4.625E-02	9.753E-03	2.471E-04	2.468E-04	4.209E-07	2.834E-06
4.750E-02	9.752E-03	2.477E-04	2.473E-04	4.383E-07	2.276E-06
4.875E-02	9.752E-03	2.481E-04	2.478E-04	4.557E-07	1.825E-06
5.000E-02	9.751E-03	2.485E-04	2.481E-04	4.732E-07	1.462E-06

TABLE 32

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+01 ACT= 9.170E-02 Catalase Activity = .123 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-02	0.	0.	0.	1.250E-03
1.250E-03	9.986E-03	1.363E-05	1.363E-05	4.807E-10	1.236E-03
2.500E-03	9.973E-03	2.725E-05	2.724E-05	1.922E-09	1.223E-03
3.750E-03	9.959E-03	4.083E-05	4.083E-05	4.322E-09	1.209E-03
5.000E-03	9.946E-03	5.440E-05	5.439E-05	7.678E-09	1.196E-03
6.250E-03	9.932E-03	6.794E-05	6.793E-05	1.199E-08	1.182E-03
7.500E-03	9.919E-03	8.146E-05	8.145E-05	1.726E-08	1.169E-03
8.750E-03	9.905E-03	9.496E-05	9.494E-05	2.347E-08	1.155E-03
1.000E-02	9.892E-03	1.084E-04	1.084E-04	3.064E-08	1.142E-03
1.125E-02	9.878E-03	1.219E-04	1.218E-04	3.876E-08	1.128E-03
1.250E-02	9.865E-03	1.353E-04	1.353E-04	4.782E-08	1.115E-03
1.375E-02	9.851E-03	1.487E-04	1.487E-04	5.783E-08	1.101E-03
1.500E-02	9.838E-03	1.621E-04	1.620E-04	6.878E-08	1.088E-03
1.625E-02	9.825E-03	1.754E-04	1.754E-04	8.068E-08	1.075E-03
1.750E-02	9.811E-03	1.888E-04	1.887E-04	9.351E-08	1.061E-03
1.875E-02	9.798E-03	2.021E-04	2.020E-04	1.073E-07	1.048E-03
2.000E-02	9.785E-03	2.153E-04	2.153E-04	1.220E-07	1.035E-03
2.125E-02	9.771E-03	2.286E-04	2.285E-04	1.376E-07	1.021E-03
2.250E-02	9.758E-03	2.418E-04	2.417E-04	1.542E-07	1.008E-03
2.375E-02	9.745E-03	2.550E-04	2.549E-04	1.717E-07	9.949E-04
2.500E-02	9.732E-03	2.682E-04	2.681E-04	1.901E-07	9.818E-04
2.625E-02	9.719E-03	2.814E-04	2.812E-04	2.095E-07	9.686E-04
2.750E-02	9.705E-03	2.945E-04	2.943E-04	2.298E-07	9.555E-04
2.875E-02	9.692E-03	3.076E-04	3.074E-04	2.510E-07	9.424E-04
3.000E-02	9.679E-03	3.207E-04	3.205E-04	2.731E-07	9.293E-04
3.125E-02	9.666E-03	3.337E-04	3.335E-04	2.962E-07	9.162E-04
3.250E-02	9.653E-03	3.468E-04	3.465E-04	3.202E-07	9.032E-04
3.375E-02	9.640E-03	3.598E-04	3.595E-04	3.450E-07	8.902E-04
3.500E-02	9.627E-03	3.727E-04	3.724E-04	3.709E-07	8.772E-04
3.625E-02	9.614E-03	3.857E-04	3.854E-04	3.976E-07	8.643E-04
3.750E-02	9.601E-03	3.986E-04	3.983E-04	4.252E-07	8.514E-04
3.875E-02	9.588E-03	4.115E-04	4.111E-04	4.537E-07	8.385E-04
4.000E-02	9.576E-03	4.243E-04	4.240E-04	4.832E-07	8.256E-04
4.125E-02	9.563E-03	4.372E-04	4.368E-04	5.135E-07	8.128E-04
4.250E-02	9.550E-03	4.500E-04	4.496E-04	5.447E-07	7.999E-04
4.375E-02	9.537E-03	4.628E-04	4.623E-04	5.769E-07	7.872E-04
4.500E-02	9.524E-03	4.755E-04	4.750E-04	6.099E-07	7.744E-04
4.625E-02	9.512E-03	4.882E-04	4.877E-04	6.439E-07	7.617E-04
4.750E-02	9.499E-03	5.009E-04	5.004E-04	6.787E-07	7.490E-04
4.875E-02	9.486E-03	5.136E-04	5.130E-04	7.144E-07	7.363E-04
5.000E-02	9.474E-03	5.262E-04	5.256E-04	7.510E-07	7.237E-04

TABLE 33

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+01 ACT= 9.170E-02 Catalase Activity = .123 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-02	0.	0.	0.	2.500E-03
1.250E-03	9.986E-03	1.397E-05	1.397E-05	4.925E-10	2.486E-03
2.500E-03	9.972E-03	2.792E-05	2.792E-05	1.969E-09	2.472E-03
3.750E-03	9.958E-03	4.185E-05	4.185E-05	4.429E-09	2.458E-03
5.000E-03	9.944E-03	5.576E-05	5.576E-05	7.869E-09	2.444E-03
6.250E-03	9.930E-03	6.966E-05	6.965E-05	1.229E-08	2.430E-03
7.500E-03	9.916E-03	8.353E-05	8.352E-05	1.769E-08	2.416E-03
8.750E-03	9.903E-03	9.739E-05	9.737E-05	2.407E-08	2.403E-03
1.000E-02	9.889E-03	1.112E-04	1.112E-04	3.142E-08	2.389E-03
1.125E-02	9.875E-03	1.250E-04	1.250E-04	3.975E-08	2.375E-03
1.250E-02	9.861E-03	1.388E-04	1.388E-04	4.905E-08	2.361E-03
1.375E-02	9.847E-03	1.526E-04	1.526E-04	5.932E-08	2.347E-03
1.500E-02	9.834E-03	1.664E-04	1.663E-04	7.056E-08	2.334E-03
1.625E-02	9.820E-03	1.801E-04	1.801E-04	8.277E-08	2.320E-03
1.750E-02	9.806E-03	1.939E-04	1.938E-04	9.595E-08	2.306E-03
1.875E-02	9.792E-03	2.076E-04	2.075E-04	1.101E-07	2.292E-03
2.000E-02	9.779E-03	2.213E-04	2.212E-04	1.252E-07	2.279E-03
2.125E-02	9.765E-03	2.349E-04	2.348E-04	1.413E-07	2.265E-03
2.250E-02	9.751E-03	2.486E-04	2.485E-04	1.583E-07	2.251E-03
2.375E-02	9.738E-03	2.622E-04	2.621E-04	1.763E-07	2.238E-03
2.500E-02	9.724E-03	2.758E-04	2.757E-04	1.953E-07	2.224E-03
2.625E-02	9.711E-03	2.894E-04	2.892E-04	2.152E-07	2.211E-03
2.750E-02	9.697E-03	3.030E-04	3.028E-04	2.360E-07	2.197E-03
2.875E-02	9.683E-03	3.165E-04	3.163E-04	2.579E-07	2.183E-03
3.000E-02	9.670E-03	3.301E-04	3.299E-04	2.807E-07	2.170E-03
3.125E-02	9.656E-03	3.436E-04	3.434E-04	3.044E-07	2.156E-03
3.250E-02	9.643E-03	3.571E-04	3.568E-04	3.291E-07	2.143E-03
3.375E-02	9.629E-03	3.706E-04	3.703E-04	3.547E-07	2.129E-03
3.500E-02	9.616E-03	3.840E-04	3.838E-04	3.813E-07	2.116E-03
3.625E-02	9.602E-03	3.975E-04	3.972E-04	4.088E-07	2.102E-03
3.750E-02	9.589E-03	4.109E-04	4.106E-04	4.373E-07	2.089E-03
3.875E-02	9.576E-03	4.243E-04	4.240E-04	4.667E-07	2.076E-03
4.000E-02	9.562E-03	4.377E-04	4.373E-04	4.971E-07	2.062E-03
4.125E-02	9.549E-03	4.511E-04	4.507E-04	5.284E-07	2.049E-03
4.250E-02	9.535E-03	4.644E-04	4.640E-04	5.606E-07	2.036E-03
4.375E-02	9.522E-03	4.778E-04	4.773E-04	5.938E-07	2.022E-03
4.500E-02	9.509E-03	4.911E-04	4.906E-04	6.279E-07	2.009E-03
4.625E-02	9.495E-03	5.044E-04	5.039E-04	6.630E-07	1.996E-03
4.750E-02	9.482E-03	5.176E-04	5.171E-04	6.989E-07	1.982E-03
4.875E-02	9.469E-03	5.309E-04	5.303E-04	7.359E-07	1.969E-03
5.000E-02	9.456E-03	5.441E-04	5.436E-04	7.737E-07	1.956E-03

TABLE 34

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+01 ACT= 9.170E-02 Catalase Activity = 123 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	2.500E-04
1.250E-03	9.997E-02	2.993E-05	2.992E-05	1.067E-09	2.201E-04
2.500E-03	9.994E-02	5.769E-05	5.768E-05	4.168E-09	1.923E-04
3.750E-03	9.992E-02	8.321E-05	8.321E-05	9.148E-09	1.668E-04
5.000E-03	9.989E-02	1.065E-04	1.065E-04	1.585E-08	1.435E-04
6.250E-03	9.987E-02	1.274E-04	1.274E-04	2.410E-08	1.226E-04
7.500E-03	9.985E-02	1.461E-04	1.461E-04	3.376E-08	1.039E-04
8.750E-03	9.984E-02	1.626E-04	1.626E-04	4.465E-08	8.735E-05
1.000E-02	9.982E-02	1.770E-04	1.770E-04	5.664E-08	7.295E-05
1.125E-02	9.981E-02	1.895E-04	1.894E-04	6.957E-08	6.051E-05
1.250E-02	9.980E-02	2.001E-04	2.000E-04	8.330E-08	4.989E-05
1.375E-02	9.979E-02	2.091E-04	2.090E-04	9.773E-08	4.090E-05
1.500E-02	9.978E-02	2.166E-04	2.165E-04	1.127E-07	3.337E-05
1.625E-02	9.978E-02	2.229E-04	2.228E-04	1.282E-07	2.710E-05
1.750E-02	9.977E-02	2.280E-04	2.279E-04	1.441E-07	2.194E-05
1.875E-02	9.977E-02	2.323E-04	2.322E-04	1.604E-07	1.770E-05
2.000E-02	9.976E-02	2.357E-04	2.356E-04	1.768E-07	1.424E-05
2.125E-02	9.976E-02	2.385E-04	2.384E-04	1.936E-07	1.143E-05
2.250E-02	9.976E-02	2.408E-04	2.407E-04	2.104E-07	9.163E-06
2.375E-02	9.976E-02	2.426E-04	2.425E-04	2.275E-07	7.333E-06
2.500E-02	9.976E-02	2.441E-04	2.439E-04	2.446E-07	5.862E-06
2.625E-02	9.975E-02	2.453E-04	2.451E-04	2.619E-07	4.682E-06
2.750E-02	9.975E-02	2.462E-04	2.460E-04	2.792E-07	3.736E-06
2.875E-02	9.975E-02	2.470E-04	2.468E-04	2.965E-07	2.980E-06
3.000E-02	9.975E-02	2.476E-04	2.473E-04	3.140E-07	2.377E-06
3.125E-02	9.975E-02	2.481E-04	2.478E-04	3.314E-07	1.895E-06
3.250E-02	9.975E-02	2.484E-04	2.482E-04	3.489E-07	1.510E-06
3.375E-02	9.975E-02	2.488E-04	2.485E-04	3.664E-07	1.204E-06
3.500E-02	9.975E-02	2.490E-04	2.487E-04	3.839E-07	9.600E-07
3.625E-02	9.975E-02	2.492E-04	2.489E-04	4.015E-07	7.657E-07
3.750E-02	9.975E-02	2.493E-04	2.490E-04	4.190E-07	6.110E-07
3.875E-02	9.975E-02	2.495E-04	2.491E-04	4.366E-07	4.879E-07
4.000E-02	9.975E-02	2.496E-04	2.492E-04	4.542E-07	3.899E-07
4.125E-02	9.975E-02	2.496E-04	2.493E-04	4.717E-07	3.120E-07
4.250E-02	9.975E-02	2.497E-04	2.493E-04	4.893E-07	2.500E-07
4.375E-02	9.975E-02	2.497E-04	2.494E-04	5.069E-07	2.006E-07
4.500E-02	9.975E-02	2.498E-04	2.494E-04	5.245E-07	1.614E-07
4.625E-02	9.975E-02	2.498E-04	2.494E-04	5.420E-07	1.302E-07
4.750E-02	9.975E-02	2.498E-04	2.494E-04	5.596E-07	1.054E-07
4.875E-02	9.975E-02	2.498E-04	2.494E-04	5.772E-07	8.567E-08
5.000E-02	9.975E-02	2.499E-04	2.494E-04	5.948E-07	6.998E-08

TABLE 35

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+01 ACT= 9.170E-02 Catalase = .123 units/ml Activity

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	1.250E-03
1.250E-03	9.995E-02	5.435E-05	5.434E-05	1.918E-09	1.196E-03
2.500E-03	9.989E-02	1.082E-04	1.082E-04	7.651E-09	1.142E-03
3.750E-03	9.984E-02	1.615E-04	1.615E-04	1.716E-08	1.088E-03
5.000E-03	9.979E-02	2.143E-04	2.143E-04	3.041E-08	1.036E-03
6.250E-03	9.973E-02	2.665E-04	2.664E-04	4.736E-08	9.835E-04
7.500E-03	9.968E-02	3.180E-04	3.179E-04	6.796E-08	9.320E-04
8.750E-03	9.963E-02	3.688E-04	3.688E-04	9.217E-08	8.812E-04
1.000E-02	9.958E-02	4.190E-04	4.189E-04	1.199E-07	8.310E-04
1.125E-02	9.953E-02	4.683E-04	4.682E-04	1.512E-07	7.817E-04
1.250E-02	9.948E-02	5.168E-04	5.166E-04	1.859E-07	7.332E-04
1.375E-02	9.944E-02	5.643E-04	5.642E-04	2.240E-07	6.856E-04
1.500E-02	9.939E-02	6.109E-04	6.107E-04	2.655E-07	6.390E-04
1.625E-02	9.934E-02	6.565E-04	6.562E-04	3.101E-07	5.935E-04
1.750E-02	9.930E-02	7.009E-04	7.006E-04	3.580E-07	5.491E-04
1.875E-02	9.926E-02	7.441E-04	7.438E-04	4.089E-07	5.059E-04
2.000E-02	9.921E-02	7.859E-04	7.856E-04	4.628E-07	4.640E-04
2.125E-02	9.917E-02	8.264E-04	8.260E-04	5.196E-07	4.236E-04
2.250E-02	9.913E-02	8.653E-04	8.649E-04	5.792E-07	3.846E-04
2.375E-02	9.910E-02	9.026E-04	9.021E-04	6.415E-07	3.473E-04
2.500E-02	9.906E-02	9.381E-04	9.376E-04	7.064E-07	3.118E-04
2.625E-02	9.903E-02	9.718E-04	9.712E-04	7.737E-07	2.781E-04
2.750E-02	9.900E-02	1.003E-03	1.003E-03	8.433E-07	2.464E-04
2.875E-02	9.897E-02	1.033E-03	1.032E-03	9.150E-07	2.168E-04
3.000E-02	9.894E-02	1.061E-03	1.060E-03	9.888E-07	1.894E-04
3.125E-02	9.891E-02	1.086E-03	1.085E-03	1.064E-06	1.641E-04
3.250E-02	9.889E-02	1.109E-03	1.108E-03	1.142E-06	1.412E-04
3.375E-02	9.887E-02	1.129E-03	1.128E-03	1.221E-06	1.205E-04
3.500E-02	9.885E-02	1.148E-03	1.147E-03	1.301E-06	1.020E-04
3.625E-02	9.884E-02	1.164E-03	1.163E-03	1.382E-06	8.578E-05
3.750E-02	9.882E-02	1.178E-03	1.177E-03	1.465E-06	7.160E-05
3.875E-02	9.881E-02	1.190E-03	1.189E-03	1.548E-06	5.937E-05
4.000E-02	9.880E-02	1.201E-03	1.200E-03	1.632E-06	4.893E-05
4.125E-02	9.879E-02	1.210E-03	1.208E-03	1.717E-06	4.011E-05
4.250E-02	9.878E-02	1.217E-03	1.216E-03	1.803E-06	3.272E-05
4.375E-02	9.878E-02	1.223E-03	1.222E-03	1.889E-06	2.658E-05
4.500E-02	9.877E-02	1.228E-03	1.227E-03	1.975E-06	2.151E-05
4.625E-02	9.877E-02	1.232E-03	1.231E-03	2.062E-06	1.736E-05
4.750E-02	9.876E-02	1.236E-03	1.234E-03	2.148E-06	1.397E-05
4.875E-02	9.876E-02	1.239E-03	1.237E-03	2.236E-06	1.122E-05
5.000E-02	9.876E-02	1.241E-03	1.239E-03	2.323E-06	9.003E-06

TABLE 36

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+01 ACT= 9.170E-02 Catalase Activity = .123 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	2.500E-03
1.250E-03	9.934E-02	6.024E-05	6.024E-05	2.125E-09	2.440E-03
2.500E-03	9.868E-02	1.203E-04	1.203E-04	8.490E-09	2.380E-03
3.750E-03	9.802E-02	1.802E-04	1.802E-04	1.908E-08	2.320E-03
5.000E-03	9.736E-02	2.399E-04	2.399E-04	3.389E-08	2.260E-03
6.250E-03	9.670E-02	2.994E-04	2.994E-04	5.290E-08	2.201E-03
7.500E-03	9.604E-02	3.587E-04	3.587E-04	7.610E-08	2.141E-03
8.750E-03	9.538E-02	4.178E-04	4.177E-04	1.035E-07	2.082E-03
1.000E-02	9.472E-02	4.767E-04	4.766E-04	1.350E-07	2.023E-03
1.125E-02	9.406E-02	5.353E-04	5.352E-04	1.707E-07	1.965E-03
1.250E-02	9.341E-02	5.937E-04	5.936E-04	2.105E-07	1.906E-03
1.375E-02	9.275E-02	6.519E-04	6.517E-04	2.543E-07	1.848E-03
1.500E-02	9.209E-02	7.098E-04	7.095E-04	3.023E-07	1.790E-03
1.625E-02	9.143E-02	7.674E-04	7.671E-04	3.544E-07	1.733E-03
1.750E-02	9.078E-02	8.247E-04	8.244E-04	4.105E-07	1.675E-03
1.875E-02	9.012E-02	8.817E-04	8.814E-04	4.706E-07	1.618E-03
2.000E-02	8.946E-02	9.384E-04	9.380E-04	5.348E-07	1.561E-03
2.125E-02	8.881E-02	9.948E-04	9.943E-04	6.029E-07	1.505E-03
2.250E-02	8.815E-02	1.051E-03	1.050E-03	6.750E-07	1.449E-03
2.375E-02	8.749E-02	1.106E-03	1.106E-03	7.510E-07	1.393E-03
2.500E-02	8.684E-02	1.162E-03	1.161E-03	8.309E-07	1.338E-03
2.625E-02	8.618E-02	1.217E-03	1.216E-03	9.147E-07	1.283E-03
2.750E-02	8.553E-02	1.271E-03	1.270E-03	1.002E-06	1.229E-03
2.875E-02	8.487E-02	1.325E-03	1.324E-03	1.094E-06	1.175E-03
3.000E-02	8.422E-02	1.378E-03	1.377E-03	1.189E-06	1.122E-03
3.125E-02	8.357E-02	1.431E-03	1.430E-03	1.288E-06	1.069E-03
3.250E-02	8.292E-02	1.483E-03	1.482E-03	1.391E-06	1.016E-03
3.375E-02	8.226E-02	1.535E-03	1.534E-03	1.497E-06	9.647E-04
3.500E-02	8.161E-02	1.586E-03	1.585E-03	1.607E-06	9.136E-04
3.625E-02	8.096E-02	1.637E-03	1.635E-03	1.720E-06	8.632E-04
3.750E-02	8.031E-02	1.686E-03	1.685E-03	1.837E-06	8.136E-04
3.875E-02	7.966E-02	1.735E-03	1.734E-03	1.958E-06	7.647E-04
4.000E-02	7.902E-02	1.783E-03	1.781E-03	2.082E-06	7.167E-04
4.125E-02	7.837E-02	1.830E-03	1.828E-03	2.209E-06	6.697E-04
4.250E-02	7.772E-02	1.876E-03	1.874E-03	2.340E-06	6.236E-04
4.375E-02	7.708E-02	1.921E-03	1.919E-03	2.473E-06	5.786E-04
4.500E-02	7.643E-02	1.965E-03	1.963E-03	2.610E-06	5.347E-04
4.625E-02	7.579E-02	2.008E-03	2.005E-03	2.750E-06	4.921E-04
4.750E-02	7.515E-02	2.049E-03	2.047E-03	2.893E-06	4.508E-04
4.875E-02	7.451E-02	2.089E-03	2.086E-03	3.039E-06	4.110E-04
5.000E-02	7.387E-02	2.127E-03	2.124E-03	3.187E-06	3.727E-04

TABLE 37

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+01 AGT= 9.170E-02 Catalase = .123 units/ml Activity

T	S	HP	GL	GA	OX
0.	1.000E+00	0.	0.	0.	2.500E-04
1.250E-03	1.000E+00	3.552E-05	3.552E-05	1.273E-09	2.145E-04
2.500E-03	9.999E-01	6.741E-05	6.741E-05	4.923E-09	1.826E-04
3.750E-03	9.999E-01	9.574E-05	9.573E-05	1.069E-08	1.543E-04
5.000E-03	9.999E-01	1.206E-04	1.206E-04	1.834E-08	1.294E-04
6.250E-03	9.999E-01	1.423E-04	1.422E-04	2.762E-08	1.077E-04
7.500E-03	9.998E-01	1.609E-04	1.608E-04	3.832E-08	8.914E-05
8.750E-03	9.998E-01	1.767E-04	1.766E-04	5.024E-08	7.332E-05
1.000E-02	9.998E-01	1.900E-04	1.900E-04	6.317E-08	5.999E-05
1.125E-02	9.998E-01	2.011E-04	2.011E-04	7.697E-08	4.885E-05
1.250E-02	9.998E-01	2.104E-04	2.103E-04	9.148E-08	3.962E-05
1.375E-02	9.998E-01	2.180E-04	2.179E-04	1.066E-07	3.202E-05
1.500E-02	9.998E-01	2.242E-04	2.241E-04	1.222E-07	2.580E-05
1.625E-02	9.998E-01	2.292E-04	2.291E-04	1.382E-07	2.074E-05
1.750E-02	9.998E-01	2.333E-04	2.332E-04	1.545E-07	1.664E-05
1.875E-02	9.998E-01	2.367E-04	2.365E-04	1.710E-07	1.333E-05
2.000E-02	9.998E-01	2.393E-04	2.392E-04	1.878E-07	1.066E-05
2.125E-02	9.998E-01	2.415E-04	2.413E-04	2.047E-07	8.516E-06
2.250E-02	9.998E-01	2.432E-04	2.430E-04	2.218E-07	6.798E-06
2.375E-02	9.998E-01	2.445E-04	2.444E-04	2.390E-07	5.423E-06
2.500E-02	9.998E-01	2.456E-04	2.454E-04	2.563E-07	4.324E-06
2.625E-02	9.998E-01	2.465E-04	2.463E-04	2.736E-07	3.447E-06
2.750E-02	9.998E-01	2.472E-04	2.470E-04	2.910E-07	2.747E-06
2.875E-02	9.998E-01	2.478E-04	2.475E-04	3.084E-07	2.188E-06
3.000E-02	9.998E-01	2.482E-04	2.480E-04	3.259E-07	1.743E-06
3.125E-02	9.998E-01	2.486E-04	2.483E-04	3.434E-07	1.389E-06
3.250E-02	9.998E-01	2.489E-04	2.486E-04	3.609E-07	1.107E-06
3.375E-02	9.998E-01	2.491E-04	2.488E-04	3.784E-07	8.826E-07
3.500E-02	9.998E-01	2.492E-04	2.489E-04	3.960E-07	7.039E-07
3.625E-02	9.998E-01	2.494E-04	2.491E-04	4.135E-07	5.617E-07
3.750E-02	9.998E-01	2.495E-04	2.492E-04	4.311E-07	4.486E-07
3.875E-02	9.998E-01	2.496E-04	2.492E-04	4.487E-07	3.586E-07
4.000E-02	9.998E-01	2.497E-04	2.493E-04	4.662E-07	2.870E-07
4.125E-02	9.998E-01	2.497E-04	2.493E-04	4.838E-07	2.301E-07
4.250E-02	9.998E-01	2.498E-04	2.494E-04	5.014E-07	1.849E-07
4.375E-02	9.998E-01	2.498E-04	2.494E-04	5.190E-07	1.488E-07
4.500E-02	9.998E-01	2.498E-04	2.494E-04	5.365E-07	1.202E-07
4.625E-02	9.998E-01	2.498E-04	2.494E-04	5.541E-07	9.745E-08
4.750E-02	9.998E-01	2.499E-04	2.494E-04	5.717E-07	7.934E-08
4.875E-02	9.997E-01	2.499E-04	2.494E-04	5.893E-07	6.495E-08
5.000E-02	9.997E-01	2.499E-04	2.494E-04	6.069E-07	5.350E-08

TABLE 38

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+01 AGT= 9.170E-02 Catalase Activity = .123 units/ml

T	S	HP	GL	GA	OX
0.	1.000E+00	0.	0.	0.	1.250E-03
1.250E-03	9.999E-01	7.730E-05	7.729E-05	2.733E-09	1.173E-03
2.500E-03	9.998E-01	1.532E-04	1.532E-04	1.086E-08	1.097E-03
3.750E-03	9.998E-01	2.275E-04	2.275E-04	2.429E-08	1.023E-03
5.000E-03	9.997E-01	3.002E-04	3.001E-04	4.290E-08	9.498E-04
6.250E-03	9.996E-01	3.710E-04	3.710E-04	6.657E-08	8.790E-04
7.500E-03	9.996E-01	4.400E-04	4.399E-04	9.516E-08	8.100E-04
8.750E-03	9.995E-01	5.068E-04	5.067E-04	1.285E-07	7.431E-04
1.000E-02	9.994E-01	5.714E-04	5.713E-04	1.666E-07	6.785E-04
1.125E-02	9.994E-01	6.336E-04	6.335E-04	2.090E-07	6.163E-04
1.250E-02	9.993E-01	6.932E-04	6.930E-04	2.558E-07	5.567E-04
1.375E-02	9.992E-01	7.500E-04	7.498E-04	3.067E-07	4.999E-04
1.500E-02	9.992E-01	8.039E-04	8.036E-04	3.615E-07	4.461E-04
1.625E-02	9.991E-01	8.546E-04	8.543E-04	4.199E-07	3.954E-04
1.750E-02	9.991E-01	9.020E-04	9.016E-04	4.818E-07	3.480E-04
1.875E-02	9.991E-01	9.460E-04	9.455E-04	5.470E-07	3.040E-04
2.000E-02	9.990E-01	9.864E-04	9.859E-04	6.151E-07	2.636E-04
2.125E-02	9.990E-01	1.023E-03	1.023E-03	6.859E-07	2.267E-04
2.250E-02	9.989E-01	1.056E-03	1.056E-03	7.592E-07	1.936E-04
2.375E-02	9.989E-01	1.086E-03	1.085E-03	8.347E-07	1.640E-04
2.500E-02	9.989E-01	1.112E-03	1.111E-03	9.121E-07	1.379E-04
2.625E-02	9.989E-01	1.135E-03	1.134E-03	9.913E-07	1.151E-04
2.750E-02	9.988E-01	1.154E-03	1.154E-03	1.072E-06	9.548E-05
2.875E-02	9.988E-01	1.171E-03	1.170E-03	1.154E-06	7.871E-05
3.000E-02	9.988E-01	1.185E-03	1.184E-03	1.237E-06	6.452E-05
3.125E-02	9.988E-01	1.197E-03	1.196E-03	1.321E-06	5.263E-05
3.250E-02	9.988E-01	1.207E-03	1.206E-03	1.406E-06	4.276E-05
3.375E-02	9.988E-01	1.215E-03	1.214E-03	1.491E-06	3.460E-05
3.500E-02	9.988E-01	1.222E-03	1.221E-03	1.577E-06	2.792E-05
3.625E-02	9.988E-01	1.227E-03	1.226E-03	1.663E-06	2.247E-05
3.750E-02	9.988E-01	1.232E-03	1.230E-03	1.750E-06	1.805E-05
3.875E-02	9.988E-01	1.235E-03	1.234E-03	1.836E-06	1.447E-05
4.000E-02	9.988E-01	1.238E-03	1.237E-03	1.923E-06	1.159E-05
4.125E-02	9.988E-01	1.240E-03	1.239E-03	2.011E-06	9.267E-06
4.250E-02	9.988E-01	1.242E-03	1.241E-03	2.098E-06	7.408E-06
4.375E-02	9.988E-01	1.244E-03	1.242E-03	2.186E-06	5.918E-06
4.500E-02	9.988E-01	1.245E-03	1.243E-03	2.273E-06	4.727E-06
4.625E-02	9.988E-01	1.246E-03	1.244E-03	2.361E-06	3.776E-06
4.750E-02	9.988E-01	1.247E-03	1.245E-03	2.449E-06	3.016E-06
4.875E-02	9.988E-01	1.247E-03	1.245E-03	2.537E-06	2.411E-06
5.000E-02	9.988E-01	1.248E-03	1.246E-03	2.624E-06	1.928E-06

TABLE 39

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+01 ACT= 9.170E-02 Catalase Activity = .123 units/ml

T	S	HP	GL	GA	OX
0.	1.000E+00	0.	0.	0.	2.500E-03
1.250E-03	9.999E-01	8.999E-05	8.999E-05	3.175E-09	2.410E-03
2.500E-03	9.998E-01	1.794E-04	1.794E-04	1.268E-08	2.321E-03
3.750E-03	9.997E-01	2.683E-04	2.683E-04	2.846E-08	2.232E-03
5.000E-03	9.996E-01	3.566E-04	3.565E-04	5.049E-08	2.143E-03
6.250E-03	9.996E-01	4.442E-04	4.441E-04	7.872E-08	2.056E-03
7.500E-03	9.995E-01	5.311E-04	5.310E-04	1.131E-07	1.969E-03
8.750E-03	9.994E-01	6.173E-04	6.172E-04	1.536E-07	1.883E-03
1.000E-02	9.993E-01	7.027E-04	7.026E-04	2.001E-07	1.797E-03
1.125E-02	9.992E-01	7.873E-04	7.871E-04	2.526E-07	1.713E-03
1.250E-02	9.991E-01	8.710E-04	8.708E-04	3.111E-07	1.629E-03
1.375E-02	9.990E-01	9.537E-04	9.534E-04	3.754E-07	1.546E-03
1.500E-02	9.990E-01	1.035E-03	1.035E-03	4.455E-07	1.464E-03
1.625E-02	9.989E-01	1.116E-03	1.116E-03	5.213E-07	1.384E-03
1.750E-02	9.988E-01	1.196E-03	1.195E-03	6.028E-07	1.304E-03
1.875E-02	9.987E-01	1.274E-03	1.273E-03	6.898E-07	1.226E-03
2.000E-02	9.986E-01	1.351E-03	1.350E-03	7.823E-07	1.149E-03
2.125E-02	9.986E-01	1.426E-03	1.425E-03	8.801E-07	1.074E-03
2.250E-02	9.985E-01	1.500E-03	1.499E-03	9.832E-07	1.000E-03
2.375E-02	9.984E-01	1.572E-03	1.571E-03	1.091E-06	9.280E-04
2.500E-02	9.984E-01	1.642E-03	1.641E-03	1.205E-06	8.577E-04
2.625E-02	9.983E-01	1.710E-03	1.709E-03	1.323E-06	7.894E-04
2.750E-02	9.982E-01	1.777E-03	1.776E-03	1.446E-06	7.232E-04
2.875E-02	9.982E-01	1.841E-03	1.839E-03	1.573E-06	6.593E-04
3.000E-02	9.981E-01	1.902E-03	1.901E-03	1.705E-06	5.979E-04
3.125E-02	9.980E-01	1.961E-03	1.959E-03	1.841E-06	5.391E-04
3.250E-02	9.980E-01	2.017E-03	2.015E-03	1.981E-06	4.832E-04
3.375E-02	9.979E-01	2.069E-03	2.068E-03	2.125E-06	4.303E-04
3.500E-02	9.979E-01	2.119E-03	2.117E-03	2.273E-06	3.806E-04
3.625E-02	9.978E-01	2.165E-03	2.164E-03	2.424E-06	3.342E-04
3.750E-02	9.978E-01	2.208E-03	2.206E-03	2.578E-06	2.913E-04
3.875E-02	9.978E-01	2.248E-03	2.246E-03	2.735E-06	2.520E-04
4.000E-02	9.977E-01	2.283E-03	2.281E-03	2.894E-06	2.163E-04
4.125E-02	9.977E-01	2.315E-03	2.313E-03	3.056E-06	1.843E-04
4.250E-02	9.977E-01	2.344E-03	2.341E-03	3.220E-06	1.557E-04
4.375E-02	9.976E-01	2.369E-03	2.366E-03	3.386E-06	1.307E-04
4.500E-02	9.976E-01	2.391E-03	2.388E-03	3.554E-06	1.089E-04
4.625E-02	9.976E-01	2.409E-03	2.407E-03	3.723E-06	9.015E-05
4.750E-02	9.976E-01	2.425E-03	2.422E-03	3.893E-06	7.420E-05
4.875E-02	9.976E-01	2.439E-03	2.436E-03	4.064E-06	6.074E-05
5.000E-02	9.975E-01	2.450E-03	2.447E-03	4.236E-06	4.949E-05

TABLE 40

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E-01 ACT= 9.170E-04 Catalase Activity = 0.0 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	2.500E-04
3.750E-01	9.992E-02	8.322E-05	8.292E-05	2.943E-07	1.668E-04
7.500E-01	9.985E-02	1.461E-04	1.451E-04	1.084E-06	1.039E-04
1.125E+00	9.981E-02	1.895E-04	1.873E-04	2.227E-06	6.051E-05
1.500E+00	9.978E-02	2.166E-04	2.130E-04	3.600E-06	3.336E-05
1.875E+00	9.977E-02	2.323E-04	2.272E-04	5.107E-06	1.769E-05
2.250E+00	9.976E-02	2.408E-04	2.342E-04	6.683E-06	9.154E-06
2.625E+00	9.975E-02	2.453E-04	2.370E-04	8.291E-06	4.673E-06
3.000E+00	9.975E-02	2.476E-04	2.377E-04	9.911E-06	2.368E-06
3.375E+00	9.975E-02	2.488E-04	2.373E-04	1.153E-05	1.195E-06
3.750E+00	9.975E-02	2.494E-04	2.363E-04	1.315E-05	6.019E-07
4.125E+00	9.975E-02	2.497E-04	2.349E-04	1.475E-05	3.029E-07
4.500E+00	9.975E-02	2.498E-04	2.335E-04	1.635E-05	1.523E-07
4.875E+00	9.975E-02	2.499E-04	2.320E-04	1.794E-05	7.660E-08
5.250E+00	9.975E-02	2.500E-04	2.304E-04	1.951E-05	3.851E-08
5.625E+00	9.975E-02	2.500E-04	2.289E-04	2.108E-05	1.936E-08
6.000E+00	9.975E-02	2.500E-04	2.274E-04	2.264E-05	9.734E-09
6.375E+00	9.975E-02	2.500E-04	2.258E-04	2.418E-05	4.893E-09
6.750E+00	9.975E-02	2.500E-04	2.243E-04	2.572E-05	2.460E-09
7.125E+00	9.975E-02	2.500E-04	2.228E-04	2.724E-05	1.237E-09
7.500E+00	9.975E-02	2.500E-04	2.212E-04	2.875E-05	6.217E-10
7.875E+00	9.975E-02	2.500E-04	2.197E-04	3.026E-05	3.125E-10
8.250E+00	9.975E-02	2.500E-04	2.182E-04	3.175E-05	1.571E-10
8.625E+00	9.975E-02	2.500E-04	2.168E-04	3.324E-05	7.898E-11
9.000E+00	9.975E-02	2.500E-04	2.153E-04	3.471E-05	3.970E-11
9.375E+00	9.975E-02	2.500E-04	2.138E-04	3.617E-05	1.996E-11
9.750E+00	9.975E-02	2.500E-04	2.124E-04	3.763E-05	1.003E-11
1.012E+01	9.975E-02	2.500E-04	2.109E-04	3.907E-05	5.044E-12
1.050E+01	9.975E-02	2.500E-04	2.095E-04	4.050E-05	2.536E-12
1.087E+01	9.975E-02	2.500E-04	2.081E-04	4.193E-05	1.275E-12
1.125E+01	9.975E-02	2.500E-04	2.067E-04	4.334E-05	6.408E-13
1.162E+01	9.975E-02	2.500E-04	2.053E-04	4.474E-05	3.221E-13
1.200E+01	9.975E-02	2.500E-04	2.039E-04	4.614E-05	1.619E-13
1.237E+01	9.975E-02	2.500E-04	2.025E-04	4.753E-05	8.140E-14
1.275E+01	9.975E-02	2.500E-04	2.011E-04	4.890E-05	4.092E-14
1.313E+01	9.975E-02	2.500E-04	1.997E-04	5.027E-05	2.057E-14
1.350E+01	9.975E-02	2.500E-04	1.984E-04	5.163E-05	1.034E-14
1.387E+01	9.975E-02	2.500E-04	1.970E-04	5.297E-05	5.199E-15
1.425E+01	9.975E-02	2.500E-04	1.957E-04	5.431E-05	2.613E-15
1.462E+01	9.975E-02	2.500E-04	1.944E-04	5.564E-05	1.314E-15
1.500E+01	9.975E-02	2.500E-04	1.930E-04	5.696E-05	6.604E-16

TABLE 41

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E-01 ACT= 9.170E-04 Catalase Activity = 0.0 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	1.250E-03
3.750E-01	9.984E-02	1.615E-04	1.610E-04	5.521E-07	1.088E-03
7.500E-01	9.968E-02	3.180E-04	3.158E-04	2.182E-06	9.320E-04
1.125E+00	9.953E-02	4.683E-04	4.635E-04	4.843E-06	7.817E-04
1.500E+00	9.939E-02	6.110E-04	6.025E-04	8.484E-06	6.390E-04
1.875E+00	9.926E-02	7.442E-04	7.311E-04	1.304E-05	5.058E-04
2.250E+00	9.913E-02	8.654E-04	8.470E-04	1.843E-05	3.846E-04
2.625E+00	9.903E-02	9.719E-04	9.474E-04	2.456E-05	2.781E-04
3.000E+00	9.894E-02	1.061E-03	1.029E-03	3.131E-05	1.893E-04
3.375E+00	9.887E-02	1.130E-03	1.091E-03	3.855E-05	1.204E-04
3.750E+00	9.882E-02	1.178E-03	1.132E-03	4.614E-05	7.152E-05
4.125E+00	9.879E-02	1.210E-03	1.156E-03	5.395E-05	4.004E-05
4.500E+00	9.877E-02	1.229E-03	1.167E-03	6.188E-05	2.145E-05
4.875E+00	9.876E-02	1.239E-03	1.169E-03	6.985E-05	1.117E-05
5.250E+00	9.876E-02	1.244E-03	1.166E-03	7.781E-05	5.719E-06
5.625E+00	9.875E-02	1.247E-03	1.161E-03	8.575E-05	2.903E-06
6.000E+00	9.875E-02	1.249E-03	1.155E-03	9.365E-05	1.467E-06
6.375E+00	9.875E-02	1.249E-03	1.148E-03	1.015E-04	7.391E-07
6.750E+00	9.875E-02	1.250E-03	1.140E-03	1.093E-04	3.720E-07
7.125E+00	9.875E-02	1.250E-03	1.133E-03	1.171E-04	1.871E-07
7.500E+00	9.875E-02	1.250E-03	1.125E-03	1.248E-04	9.410E-08
7.875E+00	9.875E-02	1.250E-03	1.118E-03	1.324E-04	4.731E-08
8.250E+00	9.875E-02	1.250E-03	1.110E-03	1.400E-04	2.379E-08
8.625E+00	9.875E-02	1.250E-03	1.102E-03	1.475E-04	1.196E-08
9.000E+00	9.875E-02	1.250E-03	1.095E-03	1.550E-04	6.011E-09
9.375E+00	9.875E-02	1.250E-03	1.088E-03	1.625E-04	3.022E-09
9.750E+00	9.875E-02	1.250E-03	1.080E-03	1.699E-04	1.519E-09
1.012E+01	9.875E-02	1.250E-03	1.073E-03	1.772E-04	7.637E-10
1.050E+01	9.875E-02	1.250E-03	1.065E-03	1.845E-04	3.839E-10
1.087E+01	9.875E-02	1.250E-03	1.058E-03	1.917E-04	1.930E-10
1.125E+01	9.875E-02	1.250E-03	1.051E-03	1.989E-04	9.702E-11
1.162E+01	9.875E-02	1.250E-03	1.044E-03	2.061E-04	4.877E-11
1.200E+01	9.875E-02	1.250E-03	1.037E-03	2.132E-04	2.452E-11
1.237E+01	9.875E-02	1.250E-03	1.030E-03	2.202E-04	1.233E-11
1.275E+01	9.875E-02	1.250E-03	1.023E-03	2.272E-04	6.196E-12
1.313E+01	9.875E-02	1.250E-03	1.016E-03	2.342E-04	3.115E-12
1.350E+01	9.875E-02	1.250E-03	1.009E-03	2.411E-04	1.566E-12
1.387E+01	9.875E-02	1.250E-03	1.002E-03	2.479E-04	7.872E-13
1.425E+01	9.875E-02	1.250E-03	9.953E-04	2.547E-04	3.957E-13
1.462E+01	9.875E-02	1.250E-03	9.885E-04	2.615E-04	1.989E-13
1.500E+01	9.875E-02	1.250E-03	9.818E-04	2.682E-04	1.000E-13

TABLE 42

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E-01 ACT= 9.170E-04 Catalase Activity = 0.0 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	2.500E-03
3.750E-01	9.982E-02	1.802E-04	1.796E-04	6.140E-07	2.320E-03
7.500E-01	9.964E-02	3.587E-04	3.563E-04	2.443E-06	2.141E-03
1.125E+00	9.946E-02	5.354E-04	5.299E-04	5.467E-06	1.965E-03
1.500E+00	9.929E-02	7.098E-04	7.002E-04	9.663E-06	1.790E-03
1.875E+00	9.912E-02	8.818E-04	8.668E-04	1.501E-05	1.618E-03
2.250E+00	9.895E-02	1.051E-03	1.029E-03	2.148E-05	1.449E-03
2.625E+00	9.878E-02	1.217E-03	1.188E-03	2.904E-05	1.283E-03
3.000E+00	9.862E-02	1.379E-03	1.341E-03	3.767E-05	1.121E-03
3.375E+00	9.846E-02	1.535E-03	1.488E-03	4.732E-05	9.645E-04
3.750E+00	9.831E-02	1.687E-03	1.629E-03	5.795E-05	8.134E-04
4.125E+00	9.817E-02	1.831E-03	1.761E-03	6.951E-05	6.694E-04
4.500E+00	9.803E-02	1.966E-03	1.884E-03	8.195E-05	5.345E-04
4.875E+00	9.791E-02	2.089E-03	1.994E-03	9.518E-05	4.107E-04
5.250E+00	9.780E-02	2.199E-03	2.090E-03	1.091E-04	3.009E-04
5.625E+00	9.771E-02	2.292E-03	2.168E-03	1.236E-04	2.080E-04
6.000E+00	9.763E-02	2.365E-03	2.227E-03	1.386E-04	1.346E-04
6.375E+00	9.758E-02	2.419E-03	2.265E-03	1.540E-04	8.126E-05
6.750E+00	9.755E-02	2.454E-03	2.284E-03	1.695E-04	4.609E-05
7.125E+00	9.752E-02	2.475E-03	2.290E-03	1.851E-04	2.492E-05
7.500E+00	9.751E-02	2.487E-03	2.286E-03	2.007E-04	1.305E-05
7.875E+00	9.751E-02	2.493E-03	2.277E-03	2.163E-04	6.704E-06
8.250E+00	9.750E-02	2.497E-03	2.265E-03	2.318E-04	3.409E-06
8.625E+00	9.750E-02	2.498E-03	2.251E-03	2.472E-04	1.724E-06
9.000E+00	9.750E-02	2.499E-03	2.237E-03	2.625E-04	8.690E-07
9.375E+00	9.750E-02	2.500E-03	2.222E-03	2.777E-04	4.375E-07
9.750E+00	9.750E-02	2.500E-03	2.207E-03	2.928E-04	2.201E-07
1.012E+01	9.750E-02	2.500E-03	2.192E-03	3.078E-04	1.107E-07
1.050E+01	9.750E-02	2.500E-03	2.177E-03	3.227E-04	5.565E-08
1.087E+01	9.750E-02	2.500E-03	2.163E-03	3.375E-04	2.798E-08
1.125E+01	9.750E-02	2.500E-03	2.148E-03	3.522E-04	1.407E-08
1.162E+01	9.750E-02	2.500E-03	2.133E-03	3.668E-04	7.071E-09
1.200E+01	9.750E-02	2.500E-03	2.119E-03	3.813E-04	3.555E-09
1.237E+01	9.750E-02	2.500E-03	2.104E-03	3.957E-04	1.787E-09
1.275E+01	9.750E-02	2.500E-03	2.090E-03	4.100E-04	8.983E-10
1.313E+01	9.750E-02	2.500E-03	2.076E-03	4.242E-04	4.516E-10
1.350E+01	9.750E-02	2.500E-03	2.062E-03	4.383E-04	2.270E-10
1.387E+01	9.750E-02	2.500E-03	2.048E-03	4.523E-04	1.141E-10
1.425E+01	9.750E-02	2.500E-03	2.034E-03	4.662E-04	5.737E-11
1.462E+01	9.750E-02	2.500E-03	2.020E-03	4.800E-04	2.884E-11
1.500E+01	9.750E-02	2.500E-03	2.006E-03	4.938E-04	1.450E-11

TABLE 43

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+00 ACT= 9.170E-03 Catalase Activity = 0.0 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	2.500E-04
7.500E-02	9.985E-02	1.461E-04	1.460E-04	1.296E-07	1.039E-04
1.500E-01	9.978E-02	2.166E-04	2.162E-04	4.326E-07	3.336E-05
2.250E-01	9.976E-02	2.408E-04	2.400E-04	8.071E-07	9.154E-06
3.000E-01	9.975E-02	2.476E-04	2.464E-04	1.203E-06	2.368E-06
3.750E-01	9.975E-02	2.494E-04	2.478E-04	1.605E-06	6.019E-07
4.500E-01	9.975E-02	2.498E-04	2.478E-04	2.008E-06	1.523E-07
5.250E-01	9.975E-02	2.500E-04	2.476E-04	2.410E-06	3.851E-08
6.000E-01	9.975E-02	2.500E-04	2.472E-04	2.812E-06	9.734E-09
6.750E-01	9.975E-02	2.500E-04	2.468E-04	3.213E-06	2.460E-09
7.500E-01	9.975E-02	2.500E-04	2.464E-04	3.614E-06	6.217E-10
8.250E-01	9.975E-02	2.500E-04	2.460E-04	4.014E-06	1.571E-10
9.000E-01	9.975E-02	2.500E-04	2.456E-04	4.413E-06	3.970E-11
9.750E-01	9.975E-02	2.500E-04	2.452E-04	4.812E-06	1.003E-11
1.050E+00	9.975E-02	2.500E-04	2.448E-04	5.210E-06	2.536E-12
1.125E+00	9.975E-02	2.500E-04	2.444E-04	5.607E-06	6.408E-13
1.200E+00	9.975E-02	2.500E-04	2.440E-04	6.004E-06	1.619E-13
1.275E+00	9.975E-02	2.500E-04	2.436E-04	6.400E-06	4.092E-14
1.350E+00	9.975E-02	2.500E-04	2.432E-04	6.795E-06	1.034E-14
1.425E+00	9.975E-02	2.500E-04	2.428E-04	7.190E-06	2.613E-15
1.500E+00	9.975E-02	2.500E-04	2.424E-04	7.584E-06	6.604E-16
1.575E+00	9.975E-02	2.500E-04	2.420E-04	7.978E-06	1.669E-16
1.650E+00	9.975E-02	2.500E-04	2.416E-04	8.370E-06	4.218E-17
1.725E+00	9.975E-02	2.500E-04	2.412E-04	8.763E-06	1.066E-17
1.800E+00	9.975E-02	2.500E-04	2.408E-04	9.154E-06	2.694E-18
1.875E+00	9.975E-02	2.500E-04	2.405E-04	9.545E-06	6.807E-19
1.950E+00	9.975E-02	2.500E-04	2.401E-04	9.935E-06	1.720E-19
2.025E+00	9.975E-02	2.500E-04	2.397E-04	1.033E-05	4.347E-20
2.100E+00	9.975E-02	2.500E-04	2.393E-04	1.071E-05	1.099E-20
2.175E+00	9.975E-02	2.500E-04	2.389E-04	1.110E-05	2.776E-21
2.250E+00	9.975E-02	2.500E-04	2.385E-04	1.149E-05	7.016E-22
2.325E+00	9.975E-02	2.500E-04	2.381E-04	1.188E-05	1.773E-22
2.400E+00	9.975E-02	2.500E-04	2.377E-04	1.226E-05	4.481E-23
2.475E+00	9.975E-02	2.500E-04	2.374E-04	1.265E-05	1.132E-23
2.550E+00	9.975E-02	2.500E-04	2.370E-04	1.304E-05	2.862E-24
2.625E+00	9.975E-02	2.500E-04	2.366E-04	1.342E-05	7.232E-25
2.700E+00	9.975E-02	2.500E-04	2.362E-04	1.380E-05	1.828E-25
2.775E+00	9.975E-02	2.500E-04	2.358E-04	1.419E-05	4.619E-26
2.850E+00	9.975E-02	2.500E-04	2.354E-04	1.457E-05	1.167E-26
2.925E+00	9.975E-02	2.500E-04	2.350E-04	1.495E-05	2.950E-27
3.000E+00	9.975E-02	2.500E-04	2.347E-04	1.533E-05	7.454E-28

TABLE 44

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+00 ACT= 9.170E-03 Catalase Activity = 0.0 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	1.250E-03
7.500E-02	9.968E-02	3.180E-04	3.177E-04	2.609E-07	9.320E-04
1.500E-01	9.939E-02	6.110E-04	6.100E-04	1.019E-06	6.390E-04
2.250E-01	9.913E-02	8.654E-04	8.632E-04	2.222E-06	3.846E-04
3.000E-01	9.894E-02	1.061E-03	1.057E-03	3.791E-06	1.893E-04
3.750E-01	9.882E-02	1.178E-03	1.173E-03	5.613E-06	7.152E-05
4.500E-01	9.877E-02	1.229E-03	1.221E-03	7.564E-06	2.145E-05
5.250E-01	9.876E-02	1.244E-03	1.235E-03	9.562E-06	5.719E-06
6.000E-01	9.875E-02	1.249E-03	1.237E-03	1.157E-05	1.467E-06
6.750E-01	9.875E-02	1.250E-03	1.236E-03	1.358E-05	3.720E-07
7.500E-01	9.875E-02	1.250E-03	1.234E-03	1.559E-05	9.410E-08
8.250E-01	9.875E-02	1.250E-03	1.232E-03	1.759E-05	2.379E-08
9.000E-01	9.875E-02	1.250E-03	1.230E-03	1.959E-05	6.011E-09
9.750E-01	9.875E-02	1.250E-03	1.228E-03	2.159E-05	1.519E-09
1.050E+00	9.875E-02	1.250E-03	1.226E-03	2.358E-05	3.839E-10
1.125E+00	9.875E-02	1.250E-03	1.224E-03	2.557E-05	9.702E-11
1.200E+00	9.875E-02	1.250E-03	1.222E-03	2.756E-05	2.452E-11
1.275E+00	9.875E-02	1.250E-03	1.220E-03	2.954E-05	6.196E-12
1.350E+00	9.875E-02	1.250E-03	1.218E-03	3.152E-05	1.566E-12
1.425E+00	9.875E-02	1.250E-03	1.216E-03	3.350E-05	3.957E-13
1.500E+00	9.875E-02	1.250E-03	1.215E-03	3.548E-05	1.000E-13
1.575E+00	9.875E-02	1.250E-03	1.213E-03	3.745E-05	2.527E-14
1.650E+00	9.875E-02	1.250E-03	1.211E-03	3.942E-05	6.387E-15
1.725E+00	9.875E-02	1.250E-03	1.209E-03	4.138E-05	1.614E-15
1.800E+00	9.875E-02	1.250E-03	1.207E-03	4.334E-05	4.079E-16
1.875E+00	9.875E-02	1.250E-03	1.205E-03	4.530E-05	1.031E-16
1.950E+00	9.875E-02	1.250E-03	1.203E-03	4.726E-05	2.605E-17
2.025E+00	9.875E-02	1.250E-03	1.201E-03	4.921E-05	6.583E-18
2.100E+00	9.875E-02	1.250E-03	1.199E-03	5.116E-05	1.664E-18
2.175E+00	9.875E-02	1.250E-03	1.197E-03	5.310E-05	4.204E-19
2.250E+00	9.875E-02	1.250E-03	1.195E-03	5.505E-05	1.062E-19
2.325E+00	9.875E-02	1.250E-03	1.193E-03	5.699E-05	2.685E-20
2.400E+00	9.875E-02	1.250E-03	1.191E-03	5.892E-05	6.785E-21
2.475E+00	9.875E-02	1.250E-03	1.189E-03	6.086E-05	1.715E-21
2.550E+00	9.875E-02	1.250E-03	1.187E-03	6.279E-05	4.333E-22
2.625E+00	9.875E-02	1.250E-03	1.185E-03	6.471E-05	1.095E-22
2.700E+00	9.875E-02	1.250E-03	1.183E-03	6.664E-05	2.767E-23
2.775E+00	9.875E-02	1.250E-03	1.181E-03	6.856E-05	6.993E-24
2.850E+00	9.875E-02	1.250E-03	1.180E-03	7.048E-05	1.767E-24
2.925E+00	9.875E-02	1.250E-03	1.178E-03	7.239E-05	4.466E-25
3.000E+00	9.875E-02	1.250E-03	1.176E-03	7.430E-05	1.129E-25

TABLE 45

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+00 ACT= 9.170E-03 Catalase Activity = 0.0 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	2.500E-03
7.500E-02	9.964E-02	3.587E-04	3.584E-04	2.921E-07	2.141E-03
1.500E-01	9.929E-02	7.098E-04	7.087E-04	1.160E-06	1.790E-03
2.250E-01	9.895E-02	1.051E-03	1.048E-03	2.589E-06	1.449E-03
3.000E-01	9.862E-02	1.379E-03	1.374E-03	4.559E-06	1.121E-03
3.750E-01	9.831E-02	1.687E-03	1.680E-03	7.042E-06	8.134E-04
4.500E-01	9.803E-02	1.966E-03	1.956E-03	1.000E-05	5.345E-04
5.250E-01	9.780E-02	2.199E-03	2.186E-03	1.337E-05	3.009E-04
6.000E-01	9.763E-02	2.365E-03	2.348E-03	1.706E-05	1.346E-04
6.750E-01	9.755E-02	2.454E-03	2.433E-03	2.096E-05	4.609E-05
7.500E-01	9.751E-02	2.487E-03	2.462E-03	2.494E-05	1.305E-05
8.250E-01	9.750E-02	2.497E-03	2.468E-03	2.894E-05	3.409E-06
9.000E-01	9.750E-02	2.499E-03	2.466E-03	3.295E-05	8.690E-07
9.750E-01	9.750E-02	2.500E-03	2.463E-03	3.696E-05	2.201E-07
1.050E+00	9.750E-02	2.500E-03	2.459E-03	4.095E-05	5.565E-08
1.125E+00	9.750E-02	2.500E-03	2.455E-03	4.495E-05	1.407E-08
1.200E+00	9.750E-02	2.500E-03	2.451E-03	4.893E-05	3.555E-09
1.275E+00	9.750E-02	2.500E-03	2.447E-03	5.291E-05	8.983E-10
1.350E+00	9.750E-02	2.500E-03	2.443E-03	5.688E-05	2.270E-10
1.425E+00	9.750E-02	2.500E-03	2.439E-03	6.085E-05	5.737E-11
1.500E+00	9.750E-02	2.500E-03	2.435E-03	6.481E-05	1.450E-11
1.575E+00	9.750E-02	2.500E-03	2.431E-03	6.876E-05	3.664E-12
1.650E+00	9.750E-02	2.500E-03	2.427E-03	7.270E-05	9.259E-13
1.725E+00	9.750E-02	2.500E-03	2.423E-03	7.664E-05	2.340E-13
1.800E+00	9.750E-02	2.500E-03	2.419E-03	8.058E-05	5.913E-14
1.875E+00	9.750E-02	2.500E-03	2.415E-03	8.451E-05	1.494E-14
1.950E+00	9.750E-02	2.500E-03	2.412E-03	8.843E-05	3.777E-15
2.025E+00	9.750E-02	2.500E-03	2.408E-03	9.234E-05	9.544E-16
2.100E+00	9.750E-02	2.500E-03	2.404E-03	9.625E-05	2.412E-16
2.175E+00	9.750E-02	2.500E-03	2.400E-03	1.002E-04	6.095E-17
2.250E+00	9.750E-02	2.500E-03	2.396E-03	1.040E-04	1.540E-17
2.325E+00	9.750E-02	2.500E-03	2.392E-03	1.079E-04	3.893E-18
2.400E+00	9.750E-02	2.500E-03	2.388E-03	1.118E-04	9.837E-19
2.475E+00	9.750E-02	2.500E-03	2.384E-03	1.157E-04	2.486E-19
2.550E+00	9.750E-02	2.500E-03	2.380E-03	1.196E-04	6.282E-20
2.625E+00	9.750E-02	2.500E-03	2.377E-03	1.234E-04	1.588E-20
2.700E+00	9.750E-02	2.500E-03	2.373E-03	1.273E-04	4.012E-21
2.775E+00	9.750E-02	2.500E-03	2.369E-03	1.311E-04	1.014E-21
2.850E+00	9.750E-02	2.500E-03	2.365E-03	1.350E-04	2.562E-22
2.925E+00	9.750E-02	2.500E-03	2.361E-03	1.388E-04	6.475E-23
3.000E+00	9.750E-02	2.500E-03	2.357E-03	1.427E-04	1.636E-23

TABLE 46

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+01 ACT= 9.170E-02 Catalase Activity = 0.0 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	2.500E-04
1.250E-03	9.997E-02	2.993E-05	2.992E-05	1.067E-09	2.201E-04
2.500E-03	9.994E-02	5.769E-05	5.768E-05	4.168E-09	1.923E-04
3.750E-03	9.992E-02	8.322E-05	8.321E-05	9.148E-09	1.668E-04
5.000E-03	9.989E-02	1.065E-04	1.065E-04	1.585E-08	1.435E-04
6.250E-03	9.987E-02	1.274E-04	1.274E-04	2.410E-08	1.226E-04
7.500E-03	9.985E-02	1.461E-04	1.461E-04	3.376E-08	1.039E-04
8.750E-03	9.984E-02	1.627E-04	1.626E-04	4.465E-08	8.735E-05
1.000E-02	9.982E-02	1.771E-04	1.770E-04	5.664E-08	7.294E-05
1.125E-02	9.981E-02	1.895E-04	1.894E-04	6.957E-08	6.051E-05
1.250E-02	9.980E-02	2.001E-04	2.000E-04	8.330E-08	4.988E-05
1.375E-02	9.979E-02	2.091E-04	2.090E-04	9.773E-08	4.089E-05
1.500E-02	9.978E-02	2.166E-04	2.165E-04	1.127E-07	3.336E-05
1.625E-02	9.978E-02	2.229E-04	2.228E-04	1.282E-07	2.710E-05
1.750E-02	9.977E-02	2.281E-04	2.279E-04	1.441E-07	2.193E-05
1.875E-02	9.977E-02	2.323E-04	2.322E-04	1.603E-07	1.769E-05
2.000E-02	9.976E-02	2.358E-04	2.356E-04	1.768E-07	1.423E-05
2.125E-02	9.976E-02	2.386E-04	2.384E-04	1.936E-07	1.142E-05
2.250E-02	9.976E-02	2.408E-04	2.406E-04	2.104E-07	9.154E-06
2.375E-02	9.976E-02	2.427E-04	2.424E-04	2.275E-07	7.324E-06
2.500E-02	9.976E-02	2.441E-04	2.439E-04	2.446E-07	5.853E-06
2.625E-02	9.975E-02	2.453E-04	2.451E-04	2.619E-07	4.673E-06
2.750E-02	9.975E-02	2.463E-04	2.460E-04	2.792E-07	3.727E-06
2.875E-02	9.975E-02	2.470E-04	2.467E-04	2.965E-07	2.971E-06
3.000E-02	9.975E-02	2.476E-04	2.473E-04	3.140E-07	2.368E-06
3.125E-02	9.975E-02	2.481E-04	2.478E-04	3.314E-07	1.886E-06
3.250E-02	9.975E-02	2.485E-04	2.481E-04	3.489E-07	1.501E-06
3.375E-02	9.975E-02	2.488E-04	2.484E-04	3.664E-07	1.195E-06
3.500E-02	9.975E-02	2.490E-04	2.487E-04	3.839E-07	9.509E-07
3.625E-02	9.975E-02	2.492E-04	2.488E-04	4.015E-07	7.566E-07
3.750E-02	9.975E-02	2.494E-04	2.490E-04	4.190E-07	6.019E-07
3.875E-02	9.975E-02	2.495E-04	2.491E-04	4.366E-07	4.788E-07
4.000E-02	9.975E-02	2.496E-04	2.492E-04	4.541E-07	3.808E-07
4.125E-02	9.975E-02	2.497E-04	2.492E-04	4.717E-07	3.029E-07
4.250E-02	9.975E-02	2.498E-04	2.493E-04	4.893E-07	2.409E-07
4.375E-02	9.975E-02	2.498E-04	2.493E-04	5.068E-07	1.916E-07
4.500E-02	9.975E-02	2.498E-04	2.493E-04	5.244E-07	1.523E-07
4.625E-02	9.975E-02	2.499E-04	2.493E-04	5.420E-07	1.211E-07
4.750E-02	9.975E-02	2.499E-04	2.493E-04	5.596E-07	9.633E-08
4.875E-02	9.975E-02	2.499E-04	2.493E-04	5.771E-07	7.660E-08
5.000E-02	9.975E-02	2.499E-04	2.493E-04	5.947E-07	6.091E-08

TABLE 47

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+01 ACT= 9.170E-02 Catalase Activity = 0.0 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	1.250E-03
1.250E-03	9.995E-02	5.435E-05	5.434E-05	1.918E-09	1.196E-03
2.500E-03	9.989E-02	1.082E-04	1.082E-04	7.651E-09	1.142E-03
3.750E-03	9.984E-02	1.615E-04	1.615E-04	1.716E-08	1.088E-03
5.000E-03	9.979E-02	2.143E-04	2.143E-04	3.041E-08	1.036E-03
6.250E-03	9.973E-02	2.665E-04	2.664E-04	4.736E-08	9.835E-04
7.500E-03	9.968E-02	3.180E-04	3.179E-04	6.796E-08	9.320E-04
8.750E-03	9.963E-02	3.689E-04	3.688E-04	9.217E-08	8.811E-04
1.000E-02	9.958E-02	4.190E-04	4.189E-04	1.199E-07	8.310E-04
1.125E-02	9.953E-02	4.683E-04	4.682E-04	1.512E-07	7.817E-04
1.250E-02	9.948E-02	5.168E-04	5.166E-04	1.859E-07	7.332E-04
1.375E-02	9.944E-02	5.644E-04	5.642E-04	2.240E-07	6.856E-04
1.500E-02	9.939E-02	6.110E-04	6.107E-04	2.655E-07	6.390E-04
1.625E-02	9.934E-02	6.566E-04	6.562E-04	3.101E-07	5.934E-04
1.750E-02	9.930E-02	7.010E-04	7.006E-04	3.580E-07	5.490E-04
1.875E-02	9.926E-02	7.442E-04	7.438E-04	4.089E-07	5.058E-04
2.000E-02	9.921E-02	7.860E-04	7.856E-04	4.628E-07	4.640E-04
2.125E-02	9.917E-02	8.265E-04	8.260E-04	5.196E-07	4.235E-04
2.250E-02	9.913E-02	8.654E-04	8.648E-04	5.792E-07	3.846E-04
2.375E-02	9.910E-02	9.027E-04	9.021E-04	6.415E-07	3.473E-04
2.500E-02	9.906E-02	9.383E-04	9.376E-04	7.064E-07	3.117E-04
2.625E-02	9.903E-02	9.719E-04	9.712E-04	7.737E-07	2.781E-04
2.750E-02	9.900E-02	1.004E-03	1.003E-03	8.433E-07	2.464E-04
2.875E-02	9.897E-02	1.033E-03	1.032E-03	9.150E-07	2.167E-04
3.000E-02	9.894E-02	1.061E-03	1.060E-03	9.888E-07	1.893E-04
3.125E-02	9.891E-02	1.086E-03	1.085E-03	1.064E-06	1.641E-04
3.250E-02	9.889E-02	1.109E-03	1.108E-03	1.142E-06	1.411E-04
3.375E-02	9.887E-02	1.130E-03	1.128E-03	1.221E-06	1.204E-04
3.500E-02	9.885E-02	1.148E-03	1.147E-03	1.301E-06	1.020E-04
3.625E-02	9.884E-02	1.164E-03	1.163E-03	1.382E-06	8.570E-05
3.750E-02	9.882E-02	1.178E-03	1.177E-03	1.465E-06	7.152E-05
3.875E-02	9.881E-02	1.191E-03	1.189E-03	1.548E-06	5.930E-05
4.000E-02	9.880E-02	1.201E-03	1.200E-03	1.632E-06	4.886E-05
4.125E-02	9.879E-02	1.210E-03	1.208E-03	1.717E-06	4.004E-05
4.250E-02	9.878E-02	1.217E-03	1.216E-03	1.803E-06	3.265E-05
4.375E-02	9.878E-02	1.223E-03	1.222E-03	1.889E-06	2.651E-05
4.500E-02	9.877E-02	1.229E-03	1.227E-03	1.975E-06	2.145E-05
4.625E-02	9.877E-02	1.233E-03	1.231E-03	2.061E-06	1.730E-05
4.750E-02	9.876E-02	1.236E-03	1.234E-03	2.148E-06	1.391E-05
4.875E-02	9.876E-02	1.239E-03	1.237E-03	2.235E-06	1.117E-05
5.000E-02	9.876E-02	1.241E-03	1.239E-03	2.323E-06	8.947E-06

TABLE 48

Computer-Generated Concentration Data For The Substituents

in a Closed Glucose Oxidase Reaction System

X= 1.000E+01 ACT= 9.170E-02 Catalase Activity = 0.0 units/ml

T	S	HP	GL	GA	OX
0.	1.000E-01	0.	0.	0.	2.500E-03
1.250E-03	9.994E-02	6.024E-05	6.024E-05	2.125E-09	2.440E-03
2.500E-03	9.988E-02	1.203E-04	1.203E-04	8.490E-09	2.380E-03
3.750E-03	9.982E-02	1.802E-04	1.802E-04	1.908E-08	2.320E-03
5.000E-03	9.976E-02	2.399E-04	2.399E-04	3.389E-08	2.260E-03
6.250E-03	9.970E-02	2.994E-04	2.994E-04	5.290E-08	2.201E-03
7.500E-03	9.964E-02	3.587E-04	3.587E-04	7.610E-08	2.141E-03
8.750E-03	9.958E-02	4.178E-04	4.177E-04	1.035E-07	2.082E-03
1.000E-02	9.952E-02	4.767E-04	4.766E-04	1.350E-07	2.023E-03
1.125E-02	9.946E-02	5.354E-04	5.352E-04	1.707E-07	1.965E-03
1.250E-02	9.941E-02	5.938E-04	5.936E-04	2.105E-07	1.906E-03
1.375E-02	9.935E-02	6.519E-04	6.517E-04	2.543E-07	1.848E-03
1.500E-02	9.929E-02	7.098E-04	7.095E-04	3.023E-07	1.790E-03
1.625E-02	9.923E-02	7.675E-04	7.671E-04	3.544E-07	1.733E-03
1.750E-02	9.918E-02	8.248E-04	8.244E-04	4.105E-07	1.675E-03
1.875E-02	9.912E-02	8.818E-04	8.814E-04	4.706E-07	1.618E-03
2.000E-02	9.906E-02	9.386E-04	9.380E-04	5.348E-07	1.561E-03
2.125E-02	9.901E-02	9.949E-04	9.943E-04	6.029E-07	1.505E-03
2.250E-02	9.895E-02	1.051E-03	1.050E-03	6.749E-07	1.449E-03
2.375E-02	9.889E-02	1.107E-03	1.106E-03	7.510E-07	1.393E-03
2.500E-02	9.884E-02	1.162E-03	1.161E-03	8.309E-07	1.338E-03
2.625E-02	9.878E-02	1.217E-03	1.216E-03	9.147E-07	1.283E-03
2.750E-02	9.873E-02	1.271E-03	1.270E-03	1.002E-06	1.229E-03
2.875E-02	9.867E-02	1.325E-03	1.324E-03	1.094E-06	1.175E-03
3.000E-02	9.862E-02	1.379E-03	1.377E-03	1.189E-06	1.121E-03
3.125E-02	9.857E-02	1.431E-03	1.430E-03	1.288E-06	1.069E-03
3.250E-02	9.852E-02	1.484E-03	1.482E-03	1.391E-06	1.016E-03
3.375E-02	9.846E-02	1.535E-03	1.534E-03	1.497E-06	9.645E-04
3.500E-02	9.841E-02	1.587E-03	1.585E-03	1.607E-06	9.134E-04
3.625E-02	9.836E-02	1.637E-03	1.635E-03	1.720E-06	8.630E-04
3.750E-02	9.831E-02	1.687E-03	1.685E-03	1.837E-06	8.134E-04
3.875E-02	9.826E-02	1.735E-03	1.734E-03	1.958E-06	7.645E-04
4.000E-02	9.822E-02	1.783E-03	1.781E-03	2.082E-06	7.165E-04
4.125E-02	9.817E-02	1.831E-03	1.828E-03	2.209E-06	6.694E-04
4.250E-02	9.812E-02	1.877E-03	1.874E-03	2.340E-06	6.234E-04
4.375E-02	9.808E-02	1.922E-03	1.919E-03	2.473E-06	5.783E-04
4.500E-02	9.803E-02	1.966E-03	1.963E-03	2.610E-06	5.345E-04
4.625E-02	9.799E-02	2.008E-03	2.005E-03	2.750E-06	4.918E-04
4.750E-02	9.795E-02	2.049E-03	2.047E-03	2.893E-06	4.506E-04
4.875E-02	9.791E-02	2.089E-03	2.086E-03	3.039E-06	4.107E-04
5.000E-02	9.787E-02	2.128E-03	2.124E-03	3.187E-06	3.724E-04

APPENDIX E

MISCELLANEOUS

E.1 Glucose Oxidase Product Literature

10-77

Product No. G-6500

GLUCOSE OXIDASE

(β-D-Glucose:oxygen 1-oxidoreductase; E.C. No. 1.1.3.4)

Type V From Aspergillus niger

Lot 97C-0322

Protein Content (Biuret): 5 mg/ml

ACTIVITY

a). Non-oxygenated system:

1480 Units/ml 296,000 Units/gm Protein

b). Oxygenated system:

2750 Units/ml 550,000 Units/gm Protein

UNIT DEFINITION: One unit will oxidize 1.0 μMole of β-D-Glucose to D-Gluconic Acid and H_2O_2 per minute at pH 5.1 at 35°C. (If reaction is saturated with oxygen, the activity may increase 50-100%.)

Storage: Store at 0-5°C.

IMPURITIES

The following impurities were checked. If present, their contamination is expressed as a % of the main activity (non-oxygenated assay).

	IMPURITY	% of the GOD Activity
a).	Maltase	1.43
b).	Glycogenase	0.005
c).	α-Amylase	0.0095
d).	β-Amylase	0.058
e).	Invertase	<0.0014
f).	Galactose Oxidase	0.85

The Catalase impurity is expressed as Sigma units/ml.

g).	Catalase	9.4

NOTE: One gram of Crystalline Catalase contains approximately 40,000,000 units, so even a small trace of Catalase will be a large number of units.

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10-77

PRINCIPLE OF THE REACTION

$$\beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{D-Gluconic Acid} + H_2O_2$$

$$H_2O_2 + \underset{\text{(colorless)}}{\text{o-Dianisidine (reduced)}} \xrightarrow{POD} \underset{\text{(red color)}}{\text{o-Dianisidine (oxidized)}} + H_2O$$

Abbreviations used: GOD = Glucose Oxidase, POD = Peroxidase

ASSAY PROCEDURE

I. Reagents

A) 0.05 M Sodium Acetate buffer, pH 5.1 at 35°C.
(Add 2.84 ml Glacial Acetic Acid to approximately 900 ml H_2O. Adjust pH to 5.1 at 35°C with 30% NaOH. Add H_2O to exactly 1000.0 ml.)

B) o-Dianisidine solution (0.0021 M)
(Dissolve 13.2 mg of Product No. D-3252 in 2.0 ml H_2O. Then dilute 1.0 ml to 100.0 ml with Reagent A.)

C) β-D-Glucose solution (10% w/v).
(Dissolve 1.0 gm of Product No. G-5250 in 10.0 ml H_2O.)

D) Peroxidase (POD) solution.
(Prepare a solution of Product No. P-8250 in H_2O containing approximately 60 Purpurogallin units/ml.)

E) Glucose Oxidase (GOD) solution.
(Prepare a solution in Reagent A containing approximately 0.5 unit/ml.)

II. Procedure

Into a silica or glass cuvette (1 cm lightpath), pipette the following:

2.40 ml of Reagent B (Dye-Buffer solution).
0.50 ml of Reagent C (Glucose solution).
0.10 ml of Reagent D (Peroxidase solution).

Mix and equilibrate the above reagents to 35°C. Monitor the A_{500} vs. air until steady. Then at zero time, add:

0.10 ml of Reagent E (Glucose Oxidase solution).

Quickly mix and record the increase in A_{500} vs. air for 2-4 minutes. Plot the A_{500} vs. time and determine the maximum linear rate. This rate is used for the calculation.

NOTE: For the O_2 saturated reaction: Immediately before use, pass PURE OXYGEN through Reagent B for 5 minutes. Then add other reagents as indicated above.

CALCULATION

$$\frac{\Delta A_{500nm}/\text{minute} \times 3.1 \text{ (Reaction Mix)}}{7.5 \times \text{ml Enzyme/Reaction Mix}} = \mu\text{Molar units/ml.}$$

$$\frac{\mu\text{Molar units/ml}}{\text{mg Protein/ml}} \times 1000 = \mu\text{Molar units/gm Protein}$$

NOTE: 7.5 is the E^{mM}_{500} for the oxidized o-Dianisidine Chromophore.

E.2 Sigma Catalase Activity Assay

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Sigma Technical
BULLETIN NO. 99
February 1972

The Determination of
CATALASE
at 240 mμ.

I. Principle:

Catalase catalyzes the reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$

Since Catalase catalyzes a first-order reaction, the amount of peroxide Substrate decomposed is directly proportional to:
1. Concentration of Substrate
2. Concentration of Enzyme.

To compare activities of various preparations, assay conditions must be identical. The H_2O_2 concentrations from the start to the finish of the assay must be accurately defined. These conditions are not met by most published procedures. The assay procedure follows:

II. Reagents:

A) Enzyme Solution:
Prepare a solution containing approximately 50 Sigma Units of Catalase per ml in 0.05 M Phosphate Buffer, pH 7.0, 25°C.
Unstable; use the dilute solutions promptly.
If you are using the crystalline Catalase suspension, Stock No. C-100, prepare a 1:5000 dilution with the buffer.

B) Substrate Solution:
To: 50 ml Phosphate Buffer, 0.05 M, pH 7.0, 25°C,
Add: 0.1 ml 30% H_2O_2
Observe the OD_{240}. For reproducible results it should be between 0.550 and 0.520.
a) If higher than this range, add Buffer to decrease the OD to specified limits.
b) If lower than this range, add Peroxide to increase the concentration.

III. Procedure:

1) To a silica cuvette, 1 cm light path, at 25°C, add:
2.9 ml Substrate Solution, (Reagent B)
0.1 ml Enzyme Solution, (Reagent A)
Mix and immediately read the OD_{240}. The initial OD will exceed 0.450 and will start to decrease.

2) Note time required for OD_{240} to decrease from 0.450 to 0.400. This corresponds to the decomposition of 3.45 μmoles of H_2O_2 in the 3 ml reaction mix.

IV. Calculations:

A. Total activity in 3 ml reaction mix.

$$\text{Total Sigma Units} = \frac{3.45}{\text{Minutes required}}$$

B. Calculate back to original Catalase used (suspension or powder), to obtain Sigma units per mg.

V. Unit Definition:

One Sigma Unit will decompose one µmole of H_2O_2 per minute at pH 7.0 at 25°C, while the H_2O_2 concentration falls from 10.3 to 9.2 µmoles per ml of reaction mix. The rate of disappearance of H_2O_2 is followed by observing the rate of decrease in OD at 240 mµ.

E.3 Calculation of The Rate Constant For The Decomposition of H_2O_2 Via Catalase

According to Aebi[16], the overall rate constant, k, for the decomposition of H_2O_2 via catalase can be calculated using the equation:

$$k = k'e$$

where k' is the specific rate constant for catalase* and e represents the molar concentration of catalase.

An approximate molar concentration of catalase can be calculated by assuming that "one gram of crystalline catalase contains about 40,000,000 units . . ."** Since the catalase activity, present in the glucose oxidase preparation, is 3.7 units/ml, the concentration of catalase (in gm/ml) is:

$$\text{catalase concentration (gm/ml)} = \frac{3.7 \text{ units/ml}}{4.0 \times 10^7 \text{ units/gm}} = 9.25 \times 10^{-8} \text{ gm/ml}$$

The catalase concentration can be expressed in moles/liter by dividing the catalase concentration (in gm/ml) by the molecular weight of catalase (the molecular weight of catalase is 250,000 gm/mole--see reference 5). Thus,

$$e = \frac{9.25 \times 10^{-8} \text{ gm/ml}}{250{,}000 \text{ gm/mole}} = 3.7 \times 10^{-13} \text{ mole/ml} = 3.7 \times 10^{-10} \text{ mole/l}$$

The molar concentration of catalase (e), multiplied by the specific rate constant ($k' = 3.4 \times 10^7 \text{ M}^{-1} \text{ sec}^{-1}$), yields the overall rate constant (k). For catalase, in the undiluted glucose oxidase

---

*k' for pure catalase from human erythrocytes is $3.4 \times 10^7 \text{ M}^{-1} \text{ sec}^{-1}$.

**Refer to Sigma product literature, page 138.

preparation, the overall rate constant is:

$$k = k'e = (3.4 \times 10^{7}\ M^{-1}\ sec^{-1})(3.7 \times 10^{-10}\ M) = .013\ sec^{-1}$$

$$\text{or } k = .755\ min^{-1}$$

The calculated rate constant, above, compares fairly well with the experimentally-determined rate constant.

TABLE 49

Data For Determining The Rate Constants of The Enzymatic Hydrolysis of Gluconolactone in Citric Acid/Phosphate Buffer (pH = 5.5) at 30°C

4% Enzyme Solution

Trial I		Trial II	
Time (min)	Absorbance	Time (min)	Absorbance
2	0.874	2	0.810
4	0.835	4	0.774
8	0.716	8	0.688
10	0.676	10	0.681
16	0.592	16	0.632

8% Enzyme Solution

Trial I		Trial II	
Time (min)	Absorbance	Time (min)	Absorbance
2	0.872	2	0.853
4	0.765	4	0.758
8	0.660	8	0.710
10	0.620	10	0.643
16	0.543	16	0.562

FIGURE 31. The Hydrolysis of Gluconolactone in a Citric Acid-Phosphate Buffer (pH = 5.5) at 30°C; 0% Enzyme Solution

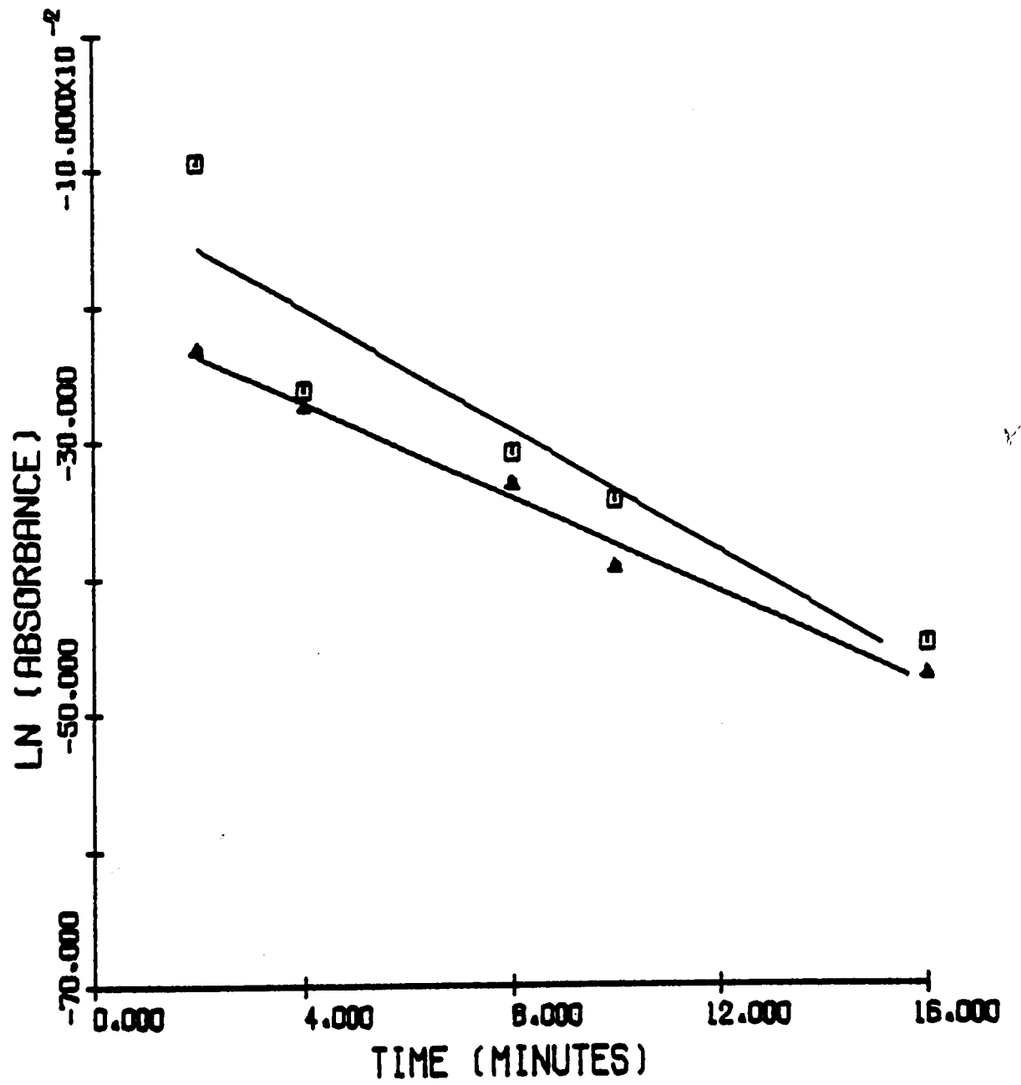

FIGURE 32. The Hydrolysis of Gluconolactone in a Citric Acid-Phosphate Buffer (pH = 5.5) at 30°C; 4% Enzyme Solution

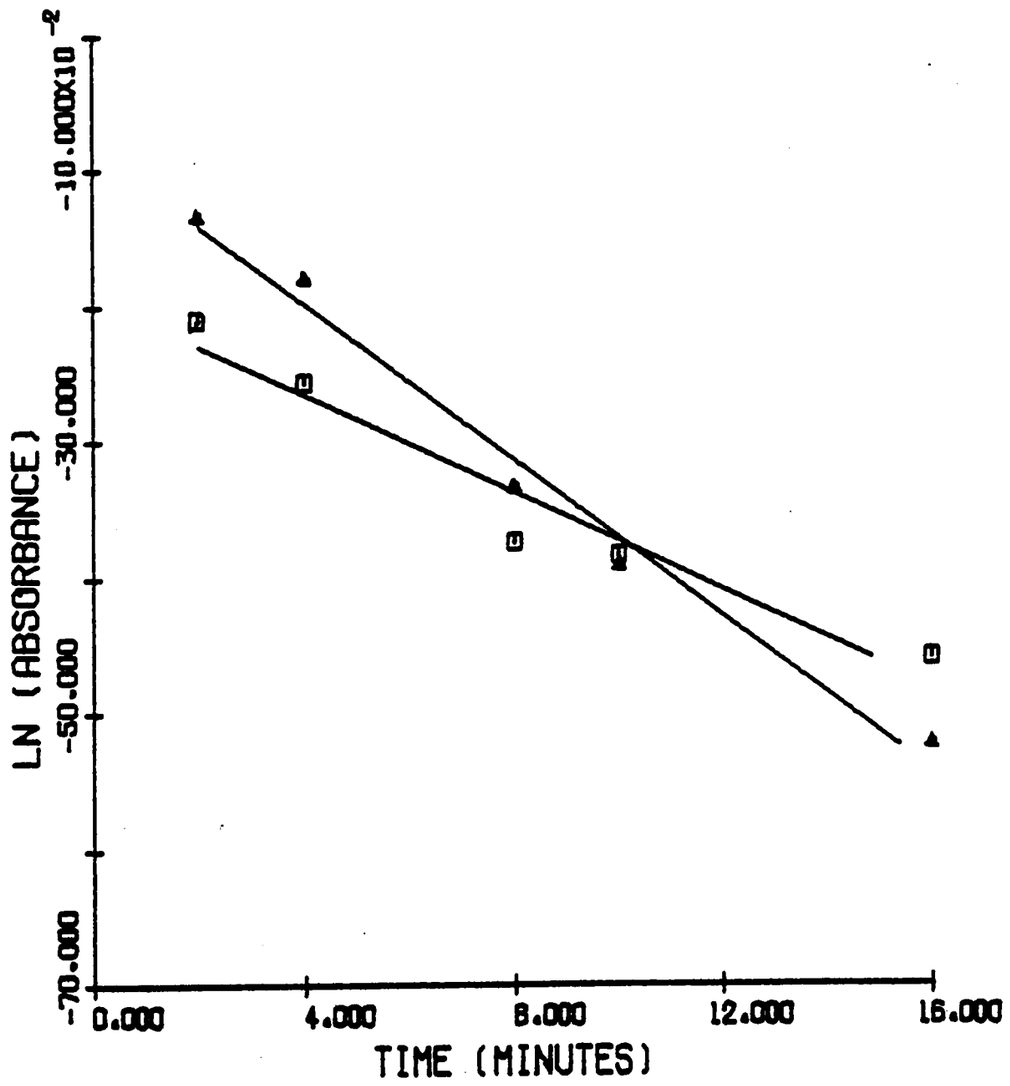

FIGURE 33. The Hydrolysis of Gluconolactone in a Citric Acid-Phosphate Buffer (pH = 5.5) at 30°C; 8% Enzyme Solution

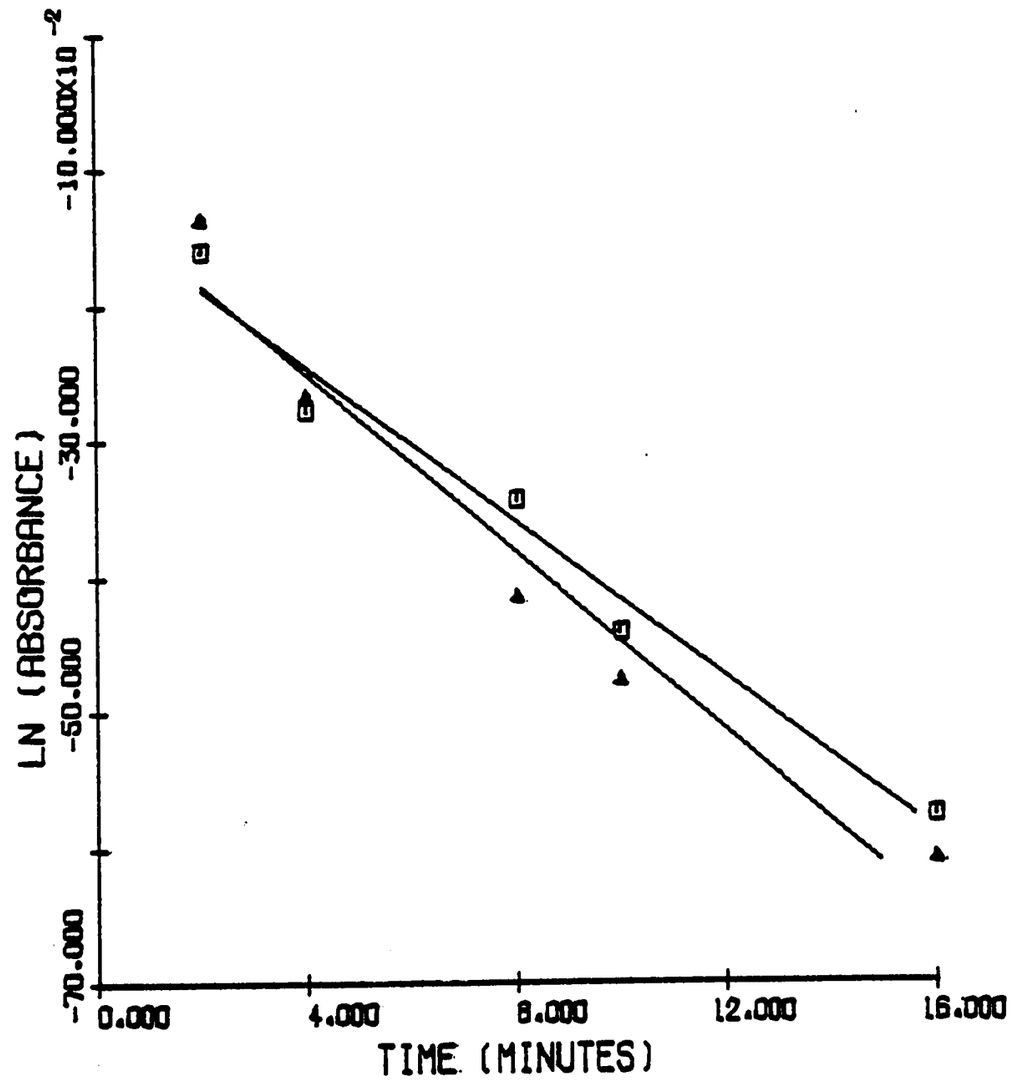

TABLE 50

Rate Constants For The Enzymatic Hydrolysis of Gluconolactone in a Citric Acid-Phosphate Buffer (pH = 5.5) at 30°C

Concentration of Enzyme	Rate Constant (min^{-1}) Experiment I	Experiment II	Average Rate Constant (min^{-1})	% Deviation From Mean
4%	.0109	.0001	.0055	98.2
8%	.0151	.0108	.0130	17.0

Note: The poor precision associated with the experiments using the 4% enzyme solution is attributed to lack of experience with the gluconolactone assay.

TABLE 51

The Conversion of Gluconolactone to Gluconic Acid

Total reaction time = 15.0 minutes.

Conversion of Gluconolactone to Gluconic Acid (%)

	Initial Glucose Concentration (M)		
Initial Oxygen Concentration (M)	.01	0.1	1.0
2.5×10^{-4}	21.6	22.8	22.9
1.25×10^{-3}	14.6	21.5	22.1
2.5×10^{-3}	12.8	19.8	21.0

Note: Quantity of glucose oxidase preparation = 0.1 ml. Activity of glucose oxidase in reactor system = .917 units/ml.

TABLE 52

The Conversion of Gluconolactone to Gluconic Acid

Total reaction time = 3.0 minutes.

Conversion of Gluconolactone to Gluconic Acid (%)

	Initial Glucose Concentration (M)		
Initial Oxygen Concentration (M)	.01	0.1	1.0
2.5×10^{-4}	6.0	6.1	6.2
1.25×10^{-3}	5.0	5.9	6.0
2.5×10^{-3}	3.7	5.7	5.9

Note: Quantity of glucose oxidase preparation = 1.0 ml. Activity of glucose oxidase in reactor system = 9.17 units/ml.

TABLE 53

The Conversion of Gluconolactone to Gluconic Acid

Total reaction time = .05 minutes (3 seconds)

Conversion of Gluconolactone to Gluconic Acid (%)

	Initial Glucose Concentration (M)		
Initial Oxygen Concentration (M)	.01	0.1	1.0
2.5×10^{-4}	.19	.24	.24
1.25×10^{-3}	.14	.19	.22
2.5×10^{-3}	.14	.15	.17

Note: Quantity of glucose oxidase preparation = 10.0 ml. Activity of glucose oxidase in reactor system = 91.7 units/ml.

TABLE 54

Comparison of Lactone Conversions For Systems With Different Glucose Oxidase Activities

The following conversions were calculated for a system having a reaction time of three minutes.

I) Conversion of Gluconolactone to Gluconic Acid (%)

	Initial Glucose Concentration (M)		
Initial Oxygen Concentration (M)	.01	0.1	1.0
2.5×10^{-4}	6.0	6.1	6.2
1.25×10^{-3}	5.0	5.9	6.0
2.5×10^{-3}	3.7	5.7	5.9

(Glucose Oxidase Activity = 9.17 units/ml)

II) Conversion of Gluconolactone to Gluconic Acid (%)

	Initial Glucose Concentration (M)		
Initial Oxygen Concentration (M)	.01	0.1	1.0
2.5×10^{-4}	3.0	4.0	5.1
1.25×10^{-3}	2.7	3.0	3.3
2.5×10^{-3}	2.7	2.7	2.8

(Glucose Oxidase Activity = .917 units/ml)

E.4 Computer Program For The Solution of The Material Balance Equations For The Glucose Oxidase Reaction System

```
RUNT VERSION FEB 74 8    18:12 06/28/79

                      SUBROUTINE INITAL
     2                COMMON /T/T,NFIN,NRUN
                     +/Y/ S,HP,GL,GA,OX
                     +/F/ DSDT,DHPDT,DGLDT,DGADT,DOXDT
                     +/PARAM/ A,B,ACT,KC,KL,IP,X,IIP,OXI,SI
     2               DATA A,B /0.5E-03, .07/
     2               REAL KC,KL
     2               IP=0
     3               IIP=41
                C
                C SET INITIAL CONDITIONS OF DEPENDENT VARIABLES
                C
     5               HP=0.0
     6               GL=0.0
     7               GA=0.0
    10               X=0.1
    11               GO TO (1,2,3,4,5,6,7,8,9) NRUN
    26          1    CONTINUE
    26               S=1.0
    30               OX=2.5E-04
    31               GO TO 10
    32          2    CONTINUE
    32               S=1.0
    34               OX=1.25E-03
    35               GO TO 10
    36          3    CONTINUE
    36               S=1.0
    40               OX=2.5E-03
    41               GO TO 10
    42          4    CONTINUE
    42               S=0.1
    44               OX=2.5E-04
    45               GO TO 10
    46          5    CONTINUE
    46               S=0.1
    50               OX=1.25E-03
    51               GO TO 10
    52          6    CONTINUE
    52               S=0.1
    54               OX=2.5E-03
    55               GO TO 10
    56          7    CONTINUE
    56               S=0.01
    60               OX=2.5E-04
    61               GO TO 10
    62          8    CONTINUE
    62               S=0.01
    64               OX=1.25E-03
    65               GO TO 10
    66          9    CONTINUE
    66               S=0.01
    70               OX=2.5E-03
    71               GO TO 10
    72         10    CONTINUE
    72               ACT=9.17E-03 * X
    74               KC=3.99E-01*X/300.0
    77               KL=0.0173 + 1.158*X/300.0
   102               OXI=OX
   104               SI=S
   105               RETURN
   106               END
```

```
                SUBROUTINE DERV
      2         COMMON /T/T,NFIN,NRUN
               +/Y/S,HP,GL,GA,OX
               +/F/ DSDT,DHPDT,DGLDT,DGADT,DOXDT
               +/PARAM/ A,B,ACT,KC,KL,IP,X,IIP,OXI,SI
      2         REAL KC,KL
      2         DSDT=-ACT/ (1.0 + A/OX +B/S)
     12         DHPDT= -DSDT -KC*HP
     15         DGLDT= -DSDT- KL*GL
     20         DGADT= KL*GL
     21         DOXDT=(KC*HP/2.0) +DSDT
     26         RETURN
     26         END


                SUBROUTINE PRINT (NI,NO)
      6         COMMON /T/T,NFIN,NRUN
               +/Y/S,HP,GL,GA,OX
               +/F/ DSDT,DHPDT,DGLDT,DGADT,DOXDT
               +/PARAM/ A,B,ACT,KC,KL,IP,X,IIP,OXI,SI
      6         REAL KC,KL
      6         DIMENSION XPLOT(50),Y1PLOT(50),Y2PLOT(50)
      6         IF(IP.GT.0) GO TO 10
     10         WRITE (NO,1000) X,ACT
     20     10  CONTINUE
     20         WRITE(NO,100) T,S,HP,GL,GA,OX
     40         IP=IP+1
     42         XPLOT(IP)=T
     45         Y1PLOT(IP)=OX
     50         Y2PLOT(IP)=GA
     53         IF (IP .LT. IIP) RETURN
     60         PUNCH 200,X,SI,OXI,(XPLOT (I), I=1,IIP)
    102         PUNCH 201,(Y1PLOT(I),I=1,IIP),(Y2PLOT(I),I=1,IIP)
    125   200   FORMAT(3(E12.5,5X),/,(6(E12.5,1X)))
    125   201   FORMAT((6(E12.5,1X)))
    125   1000  FORMAT(1H1,//,10X,*X=*,E12.3,5X,*ACT=*,E12.3,//,5X,*------T---
               +---S----------HP----------GL----------GA----------OX*,//
    125   100   FORMAT (5X,6E12.3)
    125         RETURN
    126         END
```

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